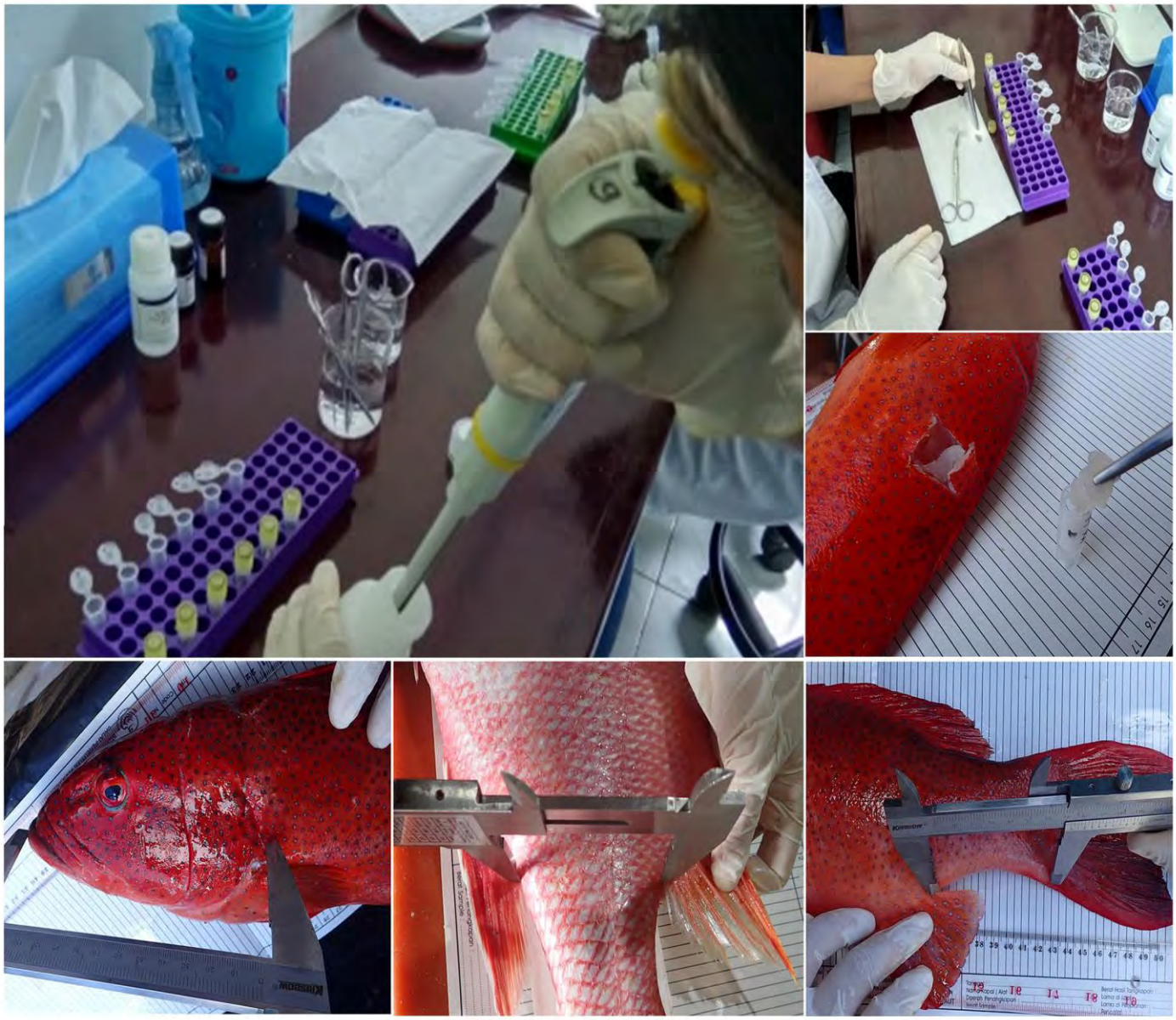

A GENETIC AND MORPHOMETRIC STUDY ON RED SNAPPER AND GROUPER IN FISHERIES MANAGEMENT AREA 715

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By

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MINISTRY OF MARINE AFFAIRS AND FISHERIES
RESEARCH INSTITUTE FOR MARINE FISHERIES
AND USAID SEA PROJECT
2021

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FOREWORD BY INDONESIAN MINISTRY OF MARINE AFFAIRS AND FISHERIES

Fish stocks in Indonesia's marine waters are important resources for the national economy and coastal communities that rely on the utilization of their livelihood resources. Natural resources of Indonesia, including fishery resources, are basic assets for the people's prosperity and should be utilized to the greatest benefit of all Indonesians. The contribution of the fishery to the economy would be optimal when fish stocks are in a healthy condition. To optimize the contribution of fishery resources to the Indonesian economy, there is a need to manage fishery resources. An appropriate management strategy is required to sustain the small pelagic fish stock and optimize economic benefit from utilizing this fishery resource, as mandated by Fisheries Act no. 31 of 2004.

To ensure that fishery management objectives can be effectively achieved, a management unit covering the whole stock unit should be identified. Consequently, it is important to delineate the boundary of the stock. The stock is biologically a natural breeding unit or population that is for the most part reproductively isolated from other such intraspecific populations. Individuals of fish in their stock may show certain phenotype characteristics, as influenced by their environment. Even though the genetic characteristics are alike, the morphological appearance of an individual may vary when living in different unique environmental conditions.

To support an effort to manage fishery targeting snapper and grouper, it is necessary to identify the stock units in FMA 715. Grouper and snapper are two groups of reef fish species targeted by fishers in Indonesia. The identification of stock units of those two families, with *Lutjanus malabaricus* and *Plectropomus leopardus* in FMA 715 as references, is carried out by implementing genetic and morphometric studies. The studies have been conducted by our researchers, as part of the commitment of MMAF - Center for Fisheries Research through Research Institute for Marine Fisheries, supported by the USAID SEA Project, to provide advice-based science to management.

This book presents the result of study on genetic and morphometric to delineate the boundary of the stock as input for effective fishery management. This study is also expected to fill the lack of information on reef fish. This study, which combines information on the stock's genetic and morphometric characters, provides a model to understand the population structure required by the fishery manager to determine stock unity for well-managed fishery.

Lastly, I would like to express my highest appreciation and gratitude to the USAID Sustainable Ecosystems Advanced Project, for supporting the research and publishing this book, the authors for conducting research and preparing this book, and many people that have provided input during preparation of the research and the book.

Yayan Hikmayani, M.Si
Head of Center for Fisheries Research
Marine and Fisheries Research and Human Resources Development Agency
Ministry of Marine Affairs and Fisheries

FOREWORD BY USAID INDONESIA

Indonesia, the world's largest archipelagic nation, is a leader in marine resource management. For over 20 years, the U.S. Government, through the United States Agency for International Development (USAID), has supported Indonesia's efforts to sustainably manage its rich marine and coastal resources. Our collaboration with the Ministry of Marine Affairs and Fisheries (MMAF) to protect marine biodiversity advances Indonesia's efforts to build a foundation for long-term economic prosperity that will benefit current and future generations and protect Indonesia's unique marine biodiversity.

MMAF's Strategic Plan for 2020-2024 seeks to advance and improve the sustainability of marine and fisheries resources to boost their contribution to the Indonesian economy as a whole. USAID supports these goals by strengthening Indonesia's self-reliance in data and scientific-based fisheries management. MMAF and USAID, through the USAID Sustainable Ecosystems Advanced project, collaboratively conducted and published this study on the *Genetic and Morphometric Study of Red Snapper (Lutjanus malabaricus) and Grouper (Plectropomus leopardus) in Fisheries Management Area 715*, both economically important species. The study concludes that those species's stocks are unique to one another and require a specific strategy and management based on the biological and fisheries characteristics -- and that doing so would enhance the effectiveness of sustainable fisheries management in the area.

Given the wealth of Indonesian fisheries resources, ensuring sustainability through improved fisheries governance will improve livelihoods and long-term socioeconomic benefits for coastal communities--and sustainable prosperity across Indonesia. USAID is proud to partner with Indonesia to advance our shared goals to strengthen sustainable fisheries.

Matthew Burton
Office Director
Environment Office
USAID Indonesia

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A Genetic and Morphometric Study on Red Snapper and Grouper in Fisheries Management Area 715 was prepared, and it is benefited by contribution from many people. Preparation and improvement of this work was benefited by inputs provided by senior scientists of the MMAF - Center for Fisheries Research (CFR), Research Institute for Marine Fisheries (RIMF), and USAID SEA Project through numerous discussion and workshops. Thanks are due to Professor Wudianto, Dr. Estu Nugroho, Dr. Duto Nugroho and Dr. Lilis Sadiyah from the CFR, Dr. Melta Rini Fahmi from the MMAF - Research Institute for Ornamental Fish Culture, and Dr. Fayakun Satria, Dr. Erfind Nurdin, Suwarso, Mahiswara, Tri Wahyu Budiarti, and Abdul Azim from the RIMF. This work was also benefited from a support to this study and a review to this book provided by Dr. Alan White of the USAID SEA Project. Their input, advice and support to this study are gratefully acknowledged.

The study was fully supported by a team from the Genetic Laboratory of the RIMF. Special thanks to Rino Agus Irwanto and Afrisa Novalina which assisted in data collection process from fish sample, morphometric measurements, preparation and PCR process, which included DNA extraction and purification, and amplification and electrophoresis process.

The collection of the samples in the field with various challenges required involvement of numerous researchers and enumerators from the RIMF and USAID SEA Partner. Personnel of the RIMF who should be appreciated for their involvement in the fish sample and morphometric data collection are Adi Surahman and Eny Fitrari (the collection in Tarakan), Muhamad Taufik, Pratiwi Lestari, Karsono, and Asri Patadjangi (the collection in Biak-Nabire), Tirtadanu and Darwin (the collection in Tarakan), Nur'ainun Muchlis (the collection in Kendari and Baubau), Anthony Panggabean, Dede Supiyandi, Melinda, and Effendi Tintis (the collection in Pagimana), Hariyanto and Samran (the collection in Wakatobi). Other people who deserved the appreciation are those from USAID SEA Partners (WCS Team lead by Intan Hartati and Sumitro) for fish sample and data collection in North Maluku, Hendra Daniel Metekohy from Papua State University for the collection in Raja Ampat, and other people from the RIFM, namely Fajar Alfina Nora, Marzuki, Late Ako, Sulaiman, Umaran for their contribution on morphometric and other data collection.

Lastly, special thanks to USAID SEA Project for fully support to the study, in particular Dr. Irna Sari**,** Sustainable Fisheries Adviser USAID SEA Project, for proposing this study as one of the USAID SEA activities.

GLOSSARY OF TERMS, ABBREVIATIONS, AND ACRONYMS

AMOVA	The analysis of molecular variance hierarchy
Clade	Haplotype groups
CR	Control region
DNA	Deoxyribonucleic acid
DnaSP	DNA Sequence Polymorphism is a software package for a comprehensive analysis of DNA polymorphism data
D-loop	Displacement loop
Euclidean distance	The length of a line segment between the two points
FMA	Fisheries management area
Fst	F-statistics
Genetics	The study of heredity, genes, and genetic variation in organisms
Haplotype	Haploid genotype. A haplotype can describe a pair of genes inherited together from one parent on one chromosome, or it can describe all of the genes on a chromosome that were inherited together from a single parent.
KOD FX Neo	Name of PCR enzymes
MMAF	Ministry of Marine Affairs and Fisheries
Morphometrics	Morphological characters mostly used to describe the variety phenotypic variations
mtDNA	Mitochondrial DNA
MU/MUs	Management Unit/s
n	Number of samples
NJ	Neighbor-joining
Nm	Number of migrants
PCR	Polymerase chain reaction
Pooled-sex	Male and female
RIMF	Research Institute for Marine Fisheries
SD	Standard deviation
SEA	Sustainable ecosystem advanced
SYBR	A nucleic acid stain in molecular biology
TBE	Tris-Borate-EDTA, a buffer solution containing a mixture of Tris base, boric acid and EDTA
TDKD, PRO	Name of primer
USAID	US Agency for International Development

EXECUTIVE SUMMARY

The Genetic and Morphometric Study on Red Snapper and Grouper in Fisheries Management Area 715 is carried out based on genetic and phenotypic variations, which also covers morphometric characteristics. The result of the research would be used in the delineation of fish stock unit for the purpose of the management of fishery in FMA 715. This study analyzed two reef fish species, namely Malabar blood snapper (*Lutjanus malabaricus*) and Leopard coral grouper (*Plectropomus leopardus*). The study was conducted by MMAF - Research Institute for Marine Fisheries (RIMF) supported by the USAID Sustainable Ecosystem Advanced (USAID SEA) Project.

The study was conducted from October 2017 to September 2019. Samples were collected from small scale fishers who caught the fishes in FMA 715 and landed their catch in fish landing sites or fishing villages. Sampling activities for Malabar blood snapper fish were carried out in Tarakan, Kendari-Baubau, Pagimana, Ternate, Biak-Nabire and Sorong-Raja Ampat. Meanwhile, Leopard coral grouper fish sampling was conducted in Wakatobi, Biak-Nabire, Pagimana, Ternate and Sorong-Raja Ampat

For population genetic study, the samples were analyzed through DNA extraction and isolations, Amplification and visualization of DNA fragments, and Sequence of PCR (amplification) products. Genetic separability analysis of fish populations was carried out using the analysis of molecular variance hierarchy (AMOVA). The results of population genetic analysis using mtDNA markers indicated that Malabar blood snapper and Leopard coral grouper from Pagimana, Ternate and Raja Ampat locations are from the same origin. The Malabar blood snapper and Leopard coral grouper population from Ternate are more related to Raja Ampat than Pagimana population. However, the result of this study using the mtDNA analysis cannot be used as a basis to say that *L. malabaricus* or *P. leopardus* in FMA 715 belong to the same stock. Further research needs to be undertaken to analyze genetic markers on the cell nucleus DNA.

Morphological or phenotypical characteristics of *P. leopardus* and *L. malabaricus* were analyzed by using data of eighteen morphometric characters as was done by Soliman et.al (2018). The Kruskal-Wallis test, cluster analysis and discriminant analysis were performed to identifying the group population among sampling locations. Based on the result of the analysis in the sampling location within FMA 715 (Pagimana, Ternate, and Raja Ampat), it was revealed that the population of Malabar blood snapper was separated from each other. Meanwhile, the Leopard coral grouper in those areas was composed of two distinct stocks, namely Pagimana and Ternate-Raja Ampat. The Malabar blood snapper from Kendari - Baubau has a close kinship with fish from Tarakan, it is suspected that process of transshipment was occurred from Tarakan to Kendari – Baubau

The management of the fishery targeting *Lutjanus malabaricus* and *Plectropomus leopardus* in FMA 715 should be carried out without waiting for complete scientific information. To effectively controlling fishing intensity, each provincial government should manage the fishery in its fisheries management area. To complement input control, reducing the time and location of fishing, as well as making the function of marine protected areas more effective, and elimination of illegal fishing practices should also be conducted.

1. GENERAL INTRODUCTION

1.1. BACKGROUND

Grouper and snapper are two groups of reef fish species targeted by fishers in Indonesia. Their distribution spread throughout sub-tropical and tropical waters in the Indo-West Pacific, from Australia to southern Japan and Korea (Allen, 1985; Kim et al. 2012; Newman 2002; Randall et al. 2003). Allen and Adrim (2003) identified 102 species of groupers (Serranidae) and 43 species of snappers (Lutjanidae) in coral reefs and intermingled habitats of less than 60 m deep in Indonesian archipelago.

Lutjanus malabaricus and *Plectropomus leopardus* are two of the most economically important benthic species in Indonesia's seas especially in eastern part of Indonesia, which includes Fisheries Management Area (FMA) 715. Both *Lutjanus malabaricus* and *Plectropomus leopardus* are the most popular targets of commercial fishers and continuously exploited by artisanal fisheries (Bawole et al., 2017; Ernawati & Budiarti, 2020). Both species are characterized as slow-growing and long-lived species, with low natural mortality (Pilling et al., 2000), and susceptible to fishing pressure (Fry et al., 2006); therefore, fisheries targeting those species need to be managed strictly. In order to ensure that fishery management objectives can be effectively achieved, a management unit (MU) covering the whole stock unit should be identified. Consequently, it is important to delineate the boundary of the stock.

The MU is a geographically delineated fishery resource that is based on practical or jurisdictional boundaries for operational stock assessment and fishery management, which may or may not reflect biological population structure (ICES, 2011; Cadrin et al., 2014). Donovan (1991) differs terms of "stock" into two categories, management stocks and biological stocks. Management stocks can be thought of as population units that can be successfully managed, while biological stocks separated based on genetic characteristics (Donovan, 1991). Biological stock is defined as natural breeding units or populations that are for the most part reproductively isolated from other such intraspecific populations (Smith et al., 1990). Individuals of fish in their stock may show certain phenotype characteristics, as influenced by their environment. Even though the genetic characteristics are alike, morphological appearance of an individual may vary when living in different unique environmental conditions, which affects their development phase (Heino, 2014). Phenotypic characteristics of individuals are plastic and vary in different environments even with a single genotype (Hard, 1995; Sultan & Stearns, 2005).

FMA 715 covers three seas and two bays, namely Tomini Bay, Maluku Sea, Halmahera Sea, Seram Sea and Berau Bay. Ackiss et al., 2013 reported that the oceanographic processes influenced genetic structure of *Caesio cuning* within this area. The bathymetric characteristics of the area have also an implication to the movement limitation and distribution of reef fish species, including *Lutjanus malabaricus* and *Plectropomus leopardus*. As reported by Allen (1985) and Heemstra & Randall (1993), those two species distribute in the depth ranges of 12 to 100 meters and 3 to 100 meters, respectively. Considering the depth ranges of those two species (Allen, 1985; Heemstra & Randall, 1993) and bathymetric condition of the FMA 715 (Figure 1), it is likely that the species of *Lutjanus malabaricus* and *Plectropomus leopardus* in FMA 715 consist of three stocks, namely the stocks in the areas of Tomini Bay, Halmahera and Obi Islands, and in the islands in the western part of West Papua Province.

For the purpose of the management of fishery targeting these two species, it is necessary to identify the stock units in FMA 715. The identification of stock unit of *Lutjanus malabaricus* and *Plectropomus leopardus* in FMA 715, as presented in this report, is carried out based on genetic and phenotypic variations, which also covers morphometric characteristics (Swain et al., 2005).

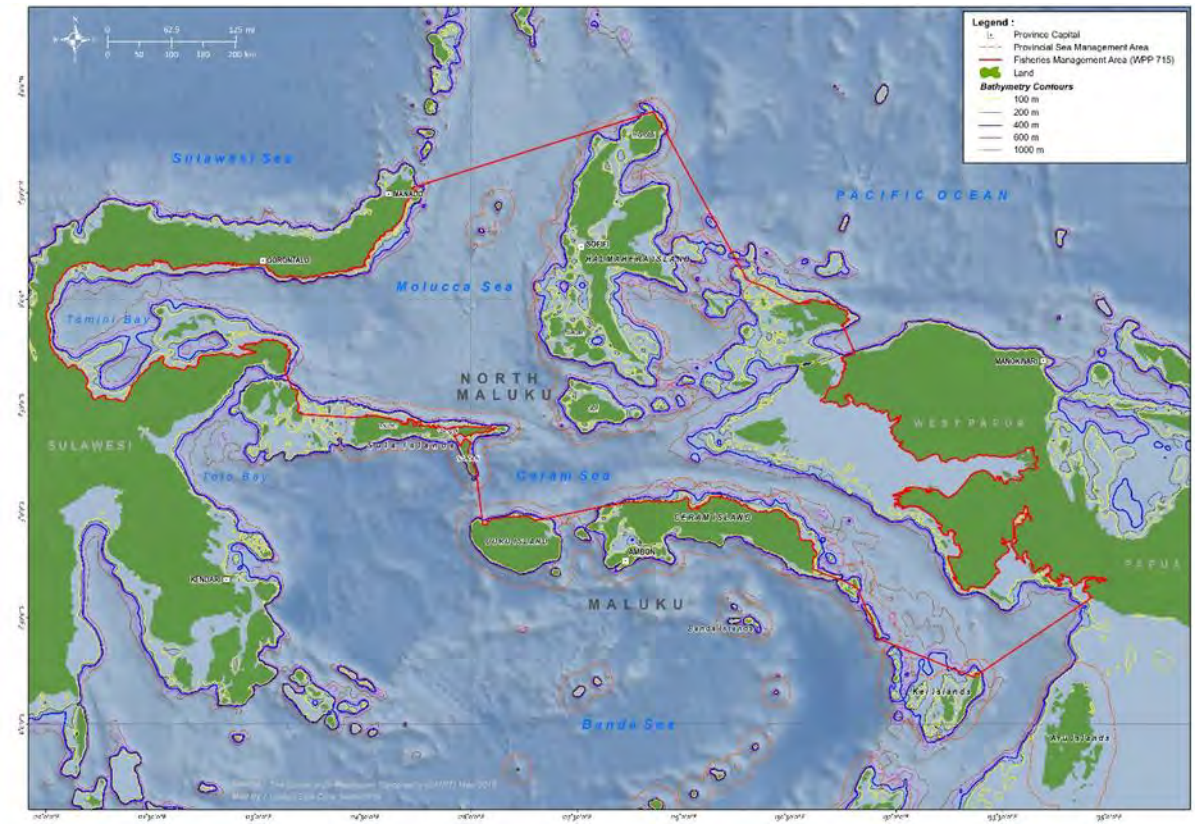

Figure 1. Indicative map of Fisheries Management Area 715

1.2. OBJECTIVES AND PURPOSE

The objectives of this study were:

1. to identify genetic and morphometric characteristics of red snapper (*Lutjanus malabaricus*) and Grouper (*Plectropomus leopardus*) in FMA 715, especially in the sampling areas of Tomini Bay, Halmahera and Obi Islands, and in the islands in the western part of West Papua Province;
2. to test the statistical different in term of genetic and morphometric characteristics of red snapper (*Lutjanus malabaricus*) and Grouper (*Plectropomus leopardus*) amongst samples from various sampling areas in FMA 715.

The result of this study would be used in the delineation of fish stock unit for the purpose of the management of fishery targeting those species in FMA 715.

1.3. IMPLEMENTATION

In accordance with the Project objectives to support the Government of Indonesia to conserve biological diversity and improve the governance of marine resources at local, district, provincial and national levels,

the USAID Sustainable Ecosystem Advanced (USAID SEA) Project supported the MMAF - Research Institute for Marine Fisheries (RIMF) to conduct this study covering from collection of the samples and data, laboratory analysis to write up of the report.

The SEA Project, implemented in West Papua, North Maluku, and Maluku Provinces, that lie within Indonesia's FMA 715, aim at (1) enhancing conservation and sustainable use of marine resources by reforming fisheries management and promoting marine protected areas to enhance fisheries productivity, food and nutrition security and sustainable livelihood within the target area; and (2) strengthening the leadership role and capacity of the Ministry of Marine Affairs and Fisheries (MMAF) and local governments to promote conservation and sustainable fishing.

2. GENERAL METHOD

2.1. SPECIES OF INTEREST

This study analyzed two reef fish species, namely Malabar blood snapper *(Lutjanus malabaricus*) and Leopard coral grouper (*Plectropomus leopardus*) (Figure 2 and Figure 3). The classification of Malabar blood snapper is as follows:

Kingdom: Animalia
Phylum: Chordata
Subphylum: Vertebrata
Superclass: Gnathostomata (Pisces)
Class: Actinopterygii
Order: Perciformes
Suborder: Percoidei
Family: Lutjanidae
Genus: Lutjanus
Species: *Lutjanus malabaricus* (Bloch & Schneider, 1801)

Figure 2. Malabar blood snapper *(Lutjanus malabaricus)*

Meanwhile, the classification of Leopard coral grouper is as follows:

Kingdom: Animalia
Phylum: Chordata
Subphylum: Vertebrata

Superclass: Gnathostomata (Pisces)
Class: Actinopterygii
Order: Perciformes
Suborder: Percoidei
Family: Serranidae
Subfamily: Epinephelinae
Genus: Plectropomus
Species: *Plectropomus leopardus* (Lacepède, 1802)

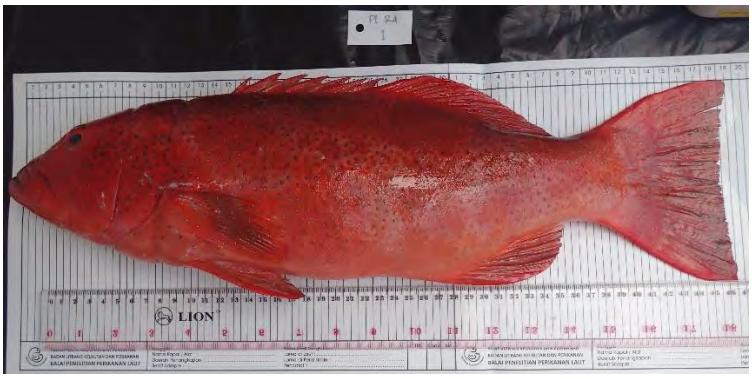

Figure 3. Leopard coral grouper *(Plectropomus leopardus)*

2.2. TIME AND LOCATION

The study was conducted from October 2017 to September 2019. Samples were collected from small scale fishers who caught the fishes in FMA 715 and landed their catch in fish landing sites or fishing villages. Sampling activities for Malabar blood snapper fish were carried out in Tarakan, Kendari-Baubau, Pagimana, Ternate, Biak-Nabire and Sorong-Raja Ampat. Meanwhile, Leopard coral grouper fish sampling was conducted in Wakatobi, Biak-Nabire, Pagimana, Ternate and Sorong-Raja Ampat (Figure 4).

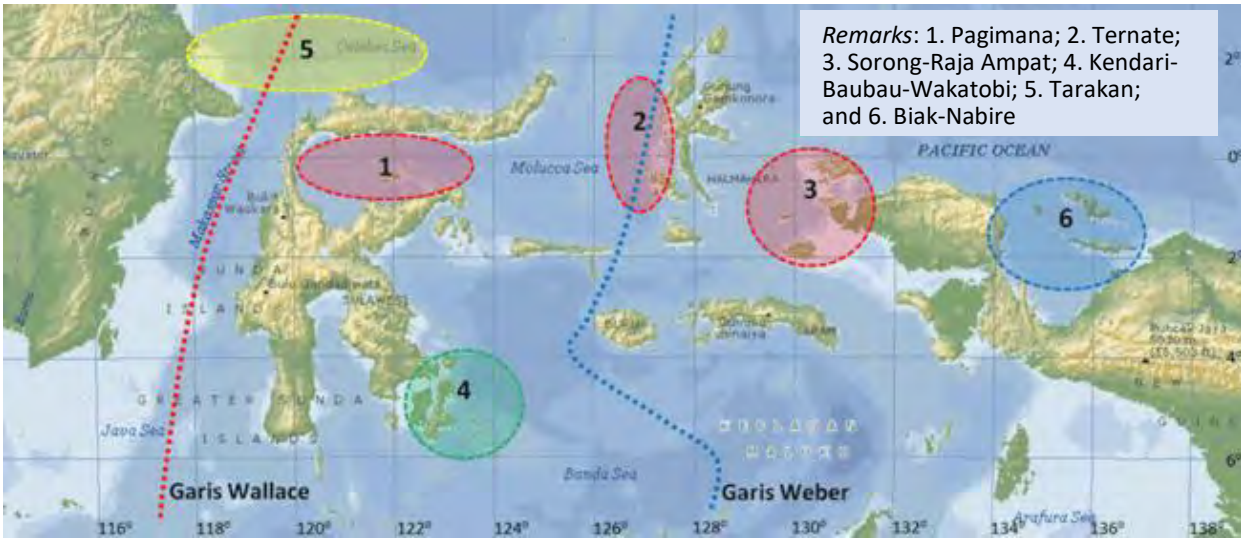

Figure 4. Map of fish sampling location

2.3. METHOD OF SAMPLING AND ANALYSIS

In the identification of the fish stock unit, this study used genetic and morphometric analyses. Activities during sampling covered identification of species, measured length and weight of individual fish samples for morphometric analysis, and prepared tissue samples of each individual fish for genetic analysis. The tissue samples were analyzed in the Genetic Laboratory of the Research Institute for Marine Fisheries (RIMF). Statistical analyses were used in the identification of the fish stock unit.

3. POPULATION GENETIC

3.1. INTRODUCTION

Lutjanus malabaricus and *Plectropomus leopardus* are economically important species in Indonesia's seas especially in eastern part of Indonesia, which includes Fisheries Management Area (FMA) 715 (Tomini Bay, Maluku Sea, Halmahera Sea, Seram Sea and Berau Bay). Both *Lutjanus malabaricus* and *Plectropomus leopardus* were the most popular targets of commercial fishers and continuously exploited by artisanal fisheries. To ensure sustainability and optimal use of the stocks of those two species, strict fisheries management is required. Therefore, stock identification is a prerequisite for the tasks of stock assessment and fishery management. Misidentification of stock can result in stock assessments that do not accurately reflect the status of the stock. An inaccurate result of stock assessment in turn may produce incorrect short-term recommendations and long-term strategy for fisheries management (ICES, 2011).

The identification of management units (MUs) is central to the management and conservation of those species and is typically used to delineate entities for monitoring and regulating the effects of human activity, especially fishery, upon the abundance of those populations (Palsbøll et al., 2007). A population, i.e. a group of organisms of the same species occupying a particular space at a particular time, may be subdivided into local populations, which are groups of interbreeding organisms (Krebs, 1972). MUs are demographically independent populations whose population dynamics depend largely on local birth and death rates rather than on immigration (Palsbøll et al., 2007). Most applied population models assume that the group of individuals has homogeneous vital rates (e.g., growth, maturity, mortality) and a closed life cycle, in which young fish in the group were produced by previous generations within the same group (Cadrin et al., 2014). Meanwhile, stock is a part of a fish population usually with a particular migration pattern, specific spawning grounds, and subject to a distinct fishery (NOAA, 2006).

There are two categories of stocks, namely management stocks, i.e. population units that can be successfully managed, and biological stocks, i.e. population units that are separated based on genetic characteristics (Donovan, 1991). Biological stock is defined as natural breeding units or populations that are for the most part reproductively isolated from other such intraspecific populations (Smith et al., 1990). For a genetic study, a working definition of stocks is provided by Ihssen et al. (1981), i.e. an intraspecific group of randomly mating individuals with temporal and spatial integrity.

Information on the genetic characteristics of a stock can be derived from the DNA in mitochondria. The mitochondria contain a single circular molecule of DNA (mtDNA) that is maternally inherited (Cadrin et al., 2014). Each mtDNA has a mitochondrial D-loop region, also called as mtDNA control region (CR). The CR is proved to be more variable sequences compared with other regions of mtDNA (Cann et al., 1984). The CR has been a useful molecular marker for revealing intraspecific genetic structure among

populations in previous research (Rosel et al., 1995), including research on the genetic diversity and population structure of *Lutjanus malabaricus* in the waters of eastern Indonesia and northern waters of Australia (Salini et al., 2006). The result of the study conducted by Salini et al. (2006) reveals the existence of two major stocks, namely central Indonesia and northern Australia stocks.

The purpose of this study is to find out the genetic variation of *L. malabaricus and P. leopardus* populations distribution in FMA 715. This information is needed to determine genetic structure of the populations, which is used to identify the stock structure of those two species in FMA 715.

3.2. METHODS

3.2.1. FISH SAMPLE COLLECTION

The study was conducted from October 2017 to September 2019. Samples of Malabar blood snapper were collected from Sorong-Raja Ampat, Biak-Nabire, Ternate, Pagimana, Kendari-Baubau, and Tarakan. Meanwhile, samples of Leopard coral grouper were collected from Sorong-Raja Ampat, Biak-Nabire, Ternate, Pagimana, and Wakatobi.

The number of samples for each species from each sampling location were 30 fishes. The tissue taken from each fish sampled was stored in a 2,000 µL micro tube and preserved with 96% ethanol. Then, the tissues were sent to the laboratory for further analysis.

3.2.2. SAMPLE ANALYSIS

a. DNA extraction and isolations

DNA isolation was carried out using Genomic DNA Mini Kit (Tissue), a product from Geneaid. Fish meat tissue stored in 96% ethanol is washed with distilled water then transfer up to 25 mg of tissue to a 1.5 ml microcentrifuge tube. Add 200 µl of GST Buffer and 20 µl of Proteinase K then vortex thoroughly. Incubate at 60°C overnight or until the sample lysate becomes clear. Furthermore, the DNA extraction method follows the instructions of the Genomic DNA Mini Kit for tissue, a product from Geneaid.

b. Amplification and visualization of DNA fragments

The amplification process (PCR) uses the KOD FX Neo mastermix, a product from Toyobo. Primer for mtDNA d-loop analysis of *L. malabaricus*: Pro889U20: CCW CTA ACT CCC AAA GCT AG and TDKD1291L21: CCT GAA ATA GGA ACC AAA TGC. Primer for mtDNA d-loop analysis of *P. leopardus*: F: AAC GGA CTC AAA CGC GAA AG and R: TCT GCC TTC TGG AGT GAA CG.

PCR reaction is carried out using a thermocycler AB brand with the following conditions:

(1) *L. malabaricus*: initial denaturation at 94 °C for 1 minute and 30 seconds, then continued with 35 repetition cycles which include 94 °C for 5 seconds, 52 °C for 30 seconds and 72 °C for 30 seconds, then the final extension at 72 °C for 5 seconds.
(2) *P. leopardus*: initial denaturation at 94 °C for 2 minutes, then continued with 35 repetition cycles which include 94 °C for 15 seconds, 55 °C for 30 seconds and 68 °C for 1 minute, then the final extension at 68 °C for 7 minutes.

PCR products were tested (visualized) using SYBR or flourosafe in a 1x TBE buffer that was run at 400 mA and 90 v conditions for 30 minutes.

c. Sequence of PCR (amplification) products

DNA sequencing is the process or technique of determining the nucleotide base sequence of a DNA molecule. This sequence is known as the DNA sequence, which is the most basic information of a gene or genome because it contains the instructions needed for the formation of the body of living things. PCR products on polyacrylamide gels of the size according to the primary design were purified by agarose-gel-cutting method followed by spin-column DNA extraction from gel. Purified PCR products are molded into PCR for sequencing using the same primary pair as the initial efficacy. This work is carried out at Singapore's First Base DNA Sequencing Service.

3.2.3. DATA ANALYSIS

Nucleotide sequencing results are edited manually based on a chromatogram. The edited nucleotide sequences are then aligned using Clustal W in the MEGA 5.2 software (Molecular Evolutionary Genetics Analysis) (Tamura et al. 2011). Phylogeny analysis of Neighbor-Joining (NJ) using MEGA 5.2 software (Tamura et al. 2011), based on the Kimura-2-paramater nucleotide substitution model with bootstrap 10,000 times. The haplotype diversity was analyzed based on the formula proposed by Nei (1987), carried out using the software DnaSP 5.10 (Librado & Rojas, 2009) and Arlequin 3.5.2.2 (Excoffier, 2015).

Genetic separability analysis of fish populations was carried out using the analysis of molecular variance hierarchy (AMOVA) in the Arlequin 3.5.2.2 program package (Excoffier, 2015). The structure of the analysis is the same as conventional F-statistics, but it is applied to the mitochondrial haplotype. The real difference for the Fst value is deduced from the zero-distribution constructed from random allocation of haplotypes to simulate populations that have the same number as the original population. Probability values are calculated with 10,000 permutation tests that guarantee to have less than 0.5% difference (Nei 1987). To explore hypotheses about the existence of a genetic structure of fish, the level of real differences from alternative population groups was analyzed using Fst in pairs of mutation differences between haplotypes. Analysis of genetic separations between populations is based on molecular differences with AMOVA, frequency of haplotypes between populations and the amount of genetic diversity. Gene flow estimates, i.e. number of migrants, (Nm) by the permutation tests with 1000 replicates with Jukes and Cantor correction, was calculated by DnaSP (Librado & Rojas, 2009).

3.3. RESULTS

3.3.1. MALABAR BLOOD SNAPPER (*Lutjanus malabaricus*)

a. Haplotype Distribution

The length of red snapper mtDNA sequences obtained from the amplification results (PCR) using TDKD and PRO primers was around 620 bp (Figure 5). The blast process with gene bank data from National Center for Biotechnology Information (www.ncbi.nlm.nih.gov) on the results of the Malabar blood snapper sequence at 6 sampling locations showed that the Biak-Nabire sample was identified as a Pinjalo pinjalo, while the samples from five other sampling locations were *Lutjanus malabaricus*. Considering the species of interest in evaluating genetic variation of red snapper is *L. malabaricus*, the samples collected from Biak-Nabire were not further analyzed.

The results of the analysis of 150 *L. malabaricus* samples from 5 study sites showed 42 haplotypes with a haplotype diversity value of 0.9170. Information about genetic information at each sampling location is presented in Table 1. The value of haplotype diversity obtained at each location was in the range of 0.7448 to 0.9172 (Table 1). The highest haplotype diversity values were found in the samples from Pagimana (0.9172), while the lowest genetic diversity values were found in the samples from Raja Ampat (0.7448). The highest number of haplotypes was found in the samples from Pagimana, which was 16 haplotypes, while the lowest number of haplotypes was found in the fish samples from Ternate, which was nine haplotypes.

Figure 5. An example of the results of the visualization of the Malabar blood snapper fish sample amplification process

Table 1. Number and diversity of haplotype, and nucleotide diversity and composition of *L. malabaricus* from each sampling area

No	Location	N Haplotype	Haplotype Diversity (h)	Nucleotide Diversity (π)	Nucleotide Composition
1	Kendari – Baubau	15	0,9064 ± 0,0395	0,0536 ± 0,0273	C: 18,39% T: 29,37% A: 38,39% G: 13,85%
2	Ternate	9	0,8552 ± 0,0455	0,0143 ± 0,0080	C: 18,30% T: 29,80% A: 38,63% G: 13,27%
3	Tarakan	10	0,8793 ± 0,0431	0,0310 ± 0,0163	C: 18,48% T: 29,24% A: 38,31% G: 13,96%
4	Raja Ampat	13	0,7448 ± 0,0826	0,0203 ± 0,0109	C: 18,34% T: 29,78% A: 38,65% G: 13,23%
5	Pagimana	16	0,9172 ± 0,0327	0,0286 ± 0,0150	C: 18,33% T: 29,75% A: 38,58% G: 13,34%

b. Analysis of Molecular Variance (AMOVA) and Gene Flow

The AMOVA test of *L. malabaricus* mtDNA D-loop revealed that 49.44% of the variation was derived from variation among populations, while 50,56% of the variation was attributed to variation within populations. The fixation index or Fst value obtained for *L. malabaricus* was 0.49443. Gene flow estimates (number of migration, Nm) obtained was about 3.7 (Nm > 1).

c. Population Genetic Analyses

A Neighbour-joining (NJ) tree was constructed using 55 haplotypes based upon 620 bp D-loop region sequences, which can identify the phylogenetic lineage and to understand the population structure. Haplotype 1 – 42 was *L. malabaricus*, while haplotype 43 – 55 was P. pinjalo. The neighbour-joining (NJ) tree shows that the haplotype of P. pinjalo separates from that of *L. malabaricus*, which indicates that the samples collected from Biak – Nabire were different species and therefore were not further analyzed.

The results of NJ tree for *L. malabaricus* species (haplotypes 1 - 42) can identify four haplotype groups (clade) at five sampling locations (Figure 6). Clade 1 is found at all sampling locations and has a high percentage (dominant) at FMA 715 locations (Ternate, Pagimana and Raja Ampat) (Figure 7). Clade 2 is only found in samples from Pagimana and Kendari-Baubau with a very low percentage, while clade 3 is also with a very low percentage and is only found in fish samples from Raja Ampat and Kendari-Baubau. Fish samples with clade 4 are found in three sampling locations, namely Tarakan, Kendari-Baubau and Pagimana, and clade 4 is dominant in fish samples from Tarakan and Kendari-Baubau.

The result of genetic distance analysis between populations shows that the lowest value is obtained from the distance between the sample from Ternate and Raja Ampat, which is 0.00161, while the highest value is found in the distance between the sample from Ternate and Tarakan, which is 0.75191 (Table 2). The genetic distance between fish samples from FMA 715 (Pagimana, Ternate and Raja Ampat), tends to have a low value (small) when compared to the genetic distance from locations outside FMA 715 (Table 2). The results of the Fst P value indicate that at the 0.05 significance level between the Pagimana, Ternate and Raja Ampat populations there were no significant differences (Table 3). Samples obtained from Tarakan differed significantly from samples from four other sampling locations, as well as samples from Kendari-Baubau.

Table 2. Genetic distances between *L. malabaricus* populations

	Kendari-Baubau	**Ternate**	**Tarakan**	**Raja Ampat**	**Pagimana**
Kendari-Baubau	0.00000				
Ternate	0.49886	0.00000			
Tarakan	0.09672	0.75191	0.00000		
Raja Ampat	0.44648	0.00161	0.70971	0.00000	
Pagimana	0.39876	0.01886	0.67430	0.04181	0.00000

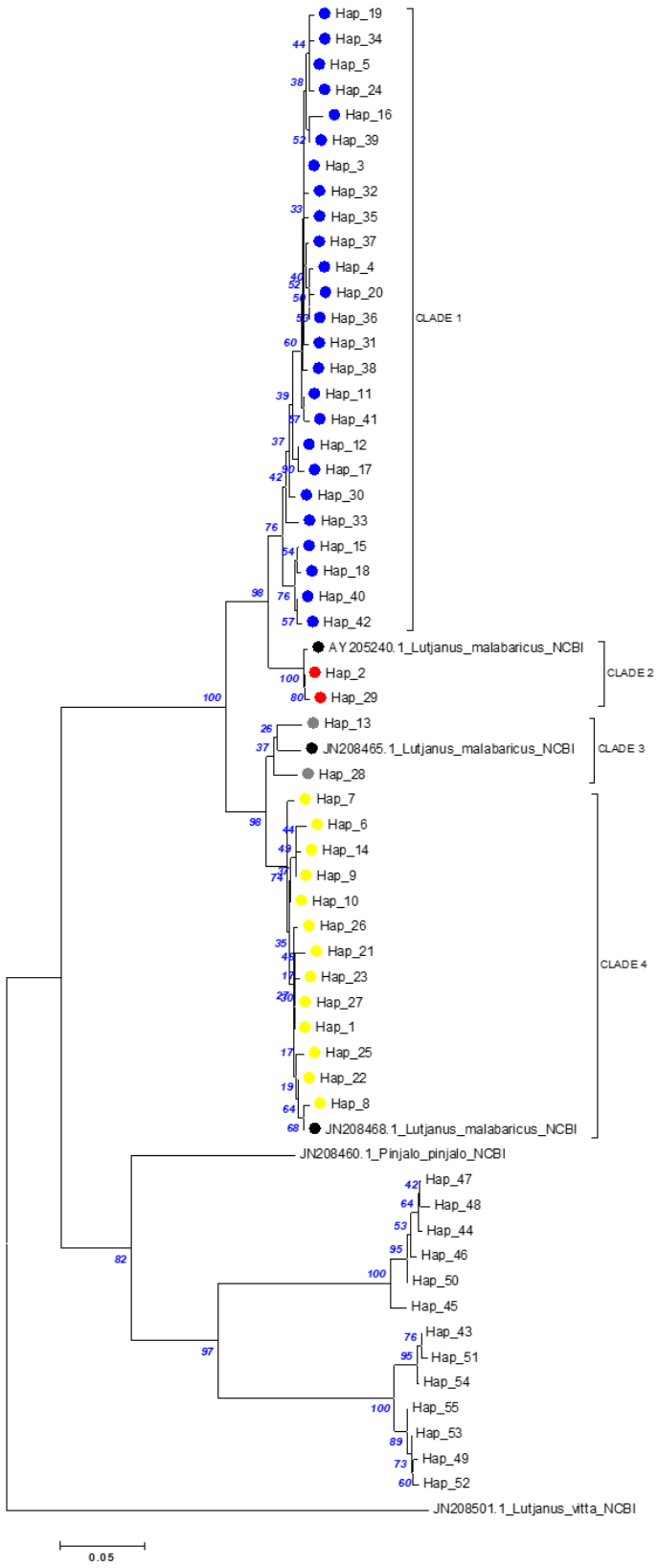

Figure 6. Rooted Neighbour-joining tree of *L. malabaricus* populations based on the mtDNA haplotypes

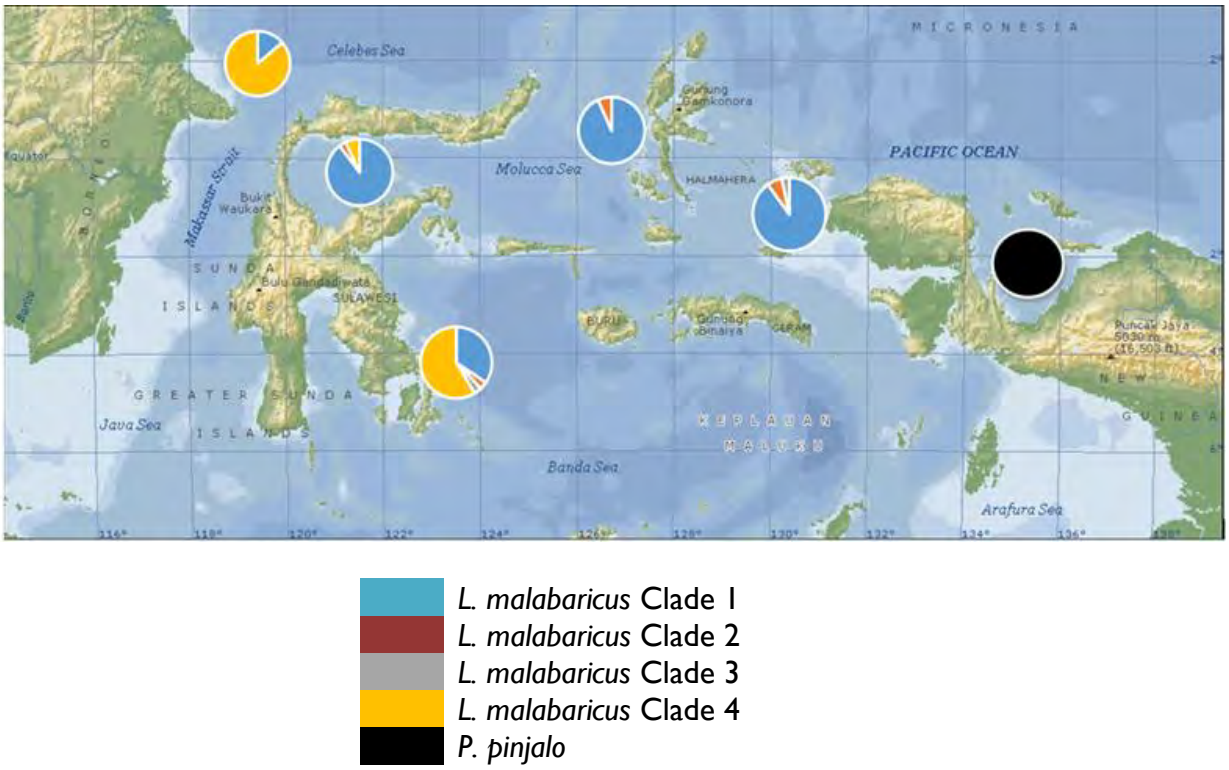

Figure 7. Overview of *L. malabaricus* population structure at 5 sampling locations

Table 3. Matrix of population differentiation p-values amongst samples of *L. malabaricus* from various location with significance level = 0.05

	Kendari-Baubau	**Ternate**	**Tarakan**	**Raja Ampat**	**Pagimana**
Kendari-Baubau					
Ternate	0.00000s ±0.0010	*			
Tarakan	0.01855s ±0.0036	0.0000s ±0.0000	*		
Raja Ampat	0.00000s ±0.0000	0.4258ns ±0.0128	0.000s ±0.000	*	
Pagimana	0.00000s ±0.0000	0.1377ns ±0.0091	0.000s ±0.000	0.0709ns ±0.0164	*

Remark: ns = not significant.

3.3.2. LEOPARD CORAL GROUPER (*Plectropomus leopardus*)

a. Haplotype Distribution

The amplification results (PCR) using mtDNA d-loop primers have a sequence length of about 490 bp (Figure 8). The results of the analysis of 150 specimens from five locations showed 58 haplotypes with a haplotype diversity value of 0.9659. Information on genetic data at each location is presented in Table 4. The value of haplotype diversity at each location was in the range of 0.8458 to 0.9890 (Table 4). The highest haplotype diversity values were obtained in samples from Ternate (0.9890), while the lowest genetic diversity values were found in samples from Biak - Nabire (0.8458). The highest number of haplotypes was obtained from the Pagimana sample, which amounted to 18 haplotypes, while the lowest number of haplotypes was obtained in the Biak-Nabire sample, which was 12 haplotypes.

Figure 8. Example of the results of the visualization of the Leopard coral grouper fish sample amplification process

Table 4. Number and diversity of haplotype, and nucleotide diversity and composition of *P. leopardus* from each sampling area

No	Location	N Haplotype	Haplotype Diversity (h)	Nucleotide Diversity (π)	Nucleotide Composition
1	Biak-Nabire	12	0,8458 ± 0,0703	0,0114 ± 0,0066	C: 17,71% T: 30,38% A: 34,71% G: 17,20%
2	Raja Ampat	13	0,9810 ± 0,0308	0,0469 ± 0,0248	C: 17,75% T: 30,21% A: 34,97% G: 17,07%
3	Wakatobi	13	0,9117 ± 0,0365	0,0381 ± 0,0196	C: 17,42% T: 30,51% A: 34,95% G: 17,13%
4	Pagimana	18	0,8621 ± 0,0632	0,0158 ± 0,0087	C: 17,67% T: 30,42% A: 34,65% G: 17,26%
5	Ternate	13	0,9890 ± 0,0314	0,0190 ± 0,0107	C: 17,76% T: 30,33% A: 34,82% G: 17,10%

b. Analysis of Molecular Variance (AMOVA) and Gene Flow

The Analysis of Molecular Variance (AMOVA) test of *L. malabaricus* mtDNA D-loop revealed that 14.26% of the variation was derived from variation among populations, while most the variation (85.74%) was attributed to variation within populations. The fixation index or Fst value obtained for *L. malabaricus* was 0.14257. Gene flow estimates (number of migration, Nm) obtained was about 2.89 (Nm > 1).

c. Population Genetic Analyses

A Neighbour-joining (NJ) tree was constructed using 58 haplotypes based upon 490 bp D-loop region sequences, which can identify the phylogenetic lineage and to understand the population structure. The results of NJ tree can identify four haplotype groups (clade) at five sampling locations (Figure 9). Clade 1 is found at all sampling locations and has a high percentage (dominant) at all sampling locations (Figure 10). Clade 2 was only found in samples from Wakatobi whose percentage was very low, Clade 3 was also very low and only in fish samples from Raja Ampat, Ternate and Wakatobi. Clade 4 fish samples were found in Raja Ampat locations with a small percentage.

The results of genetic distance analysis between populations revealed that the lowest value was obtained from the distance between samples from Ternate and Raja Ampat, which was 0.01072, while the highest value was found in the distance between samples from Wakatobi and Biak-Nabire, which was 0.20208 (Table 5). Same with the *L. malabaricus* results, the genetic distance between fish samples from FMA 715 waters (Pagimana, Ternate and Raja Ampat), has a low value (small) when compared to genetic distance outside FMA 715 waters. The results of Fst P values in *P. leopardus* have the same type as *L. malabaricus*, at the 0.05 significance level between fish from Pagimana, Ternate and Raja Ampat there was no significant difference (Table 6).

Table 5. Genetic distance between populations of *P. leopardus*

	Biak-Nabire	**Raja Ampat**	**Wakatobi**	**Pagimana**	**Ternate**
Biak-Nabire	0.00000				
Raja Ampat	0.14205	0.00000			
Wakatobi	0.20208	0.06218	0.00000		
Pagimana	0.13380	0.03854	0.18564	0.00000	
Ternate	0.15424	0.01072	0.14272	0.02579	0.00000

Table 6. Matrix of population differentiation p-values amongst samples of *P. leopardus* from various location with significance level = 0.05

	Biak-Nabire	**Raja Ampat**	**Wakatobi**	**Pagimana**	**Ternate**
Biak-Nabire	*				
Raja Ampat	0.00000^{S} ±0.0000	*			
Wakatobi	0.00000^{S} ±0.0000	0.04883^{S} ±0.0070	*		
Pagimana	0.00000^{S} ±0.0000	0.08691^{ns} ±0.0090	0.00000^{S} ±0.0000	*	
Ternate	0.00391^{S} ±0.0019	0.26855^{ns} ±0.0135	0.00098^{s} ±0.0010	0.12793^{ns} ±0.0125	*

Remark: ns = not significant.

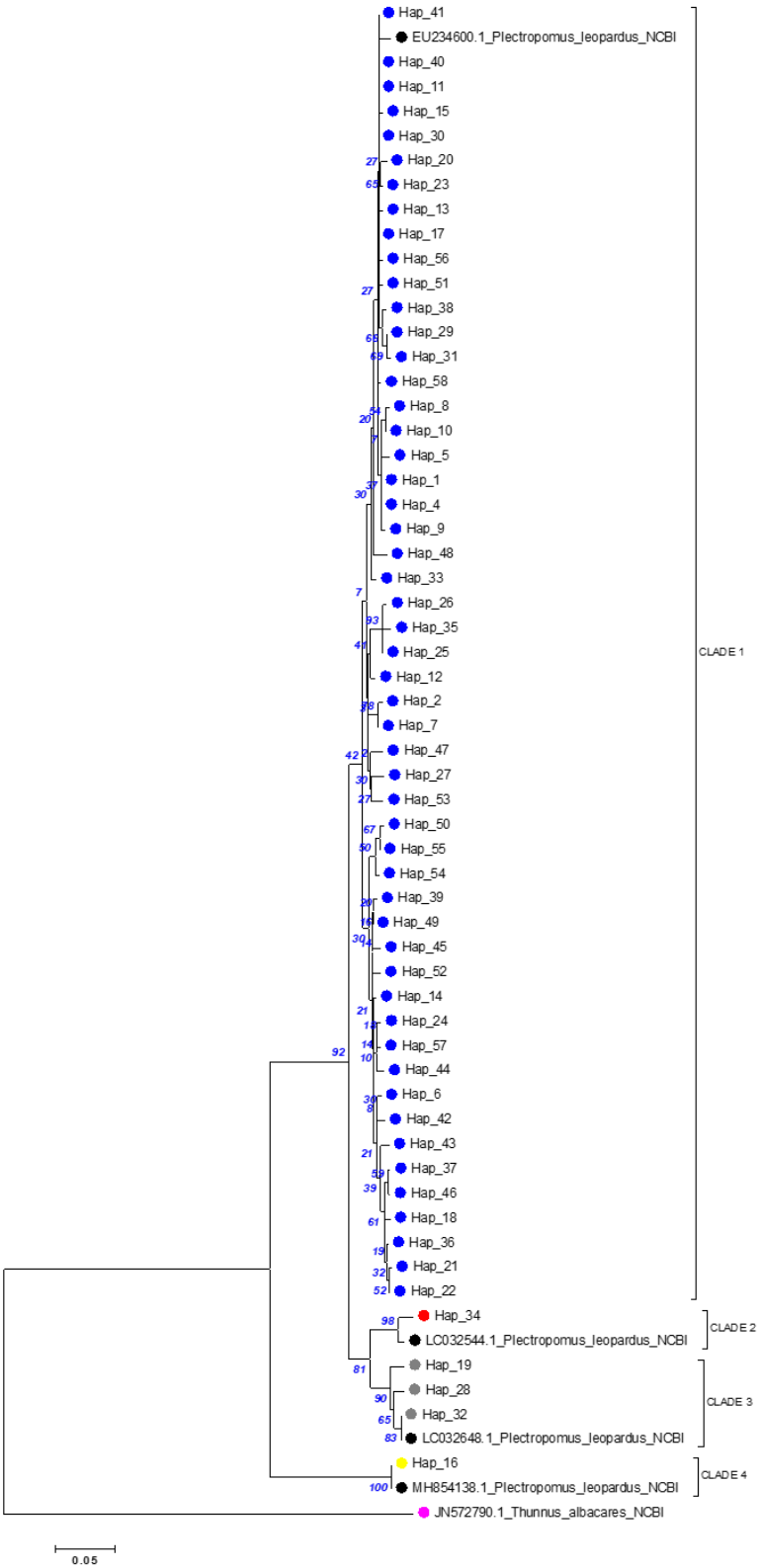

Figure 9. Rooted Neighbour-joining tree of *P. leopardus* populations based on the mtDNA haplotypes

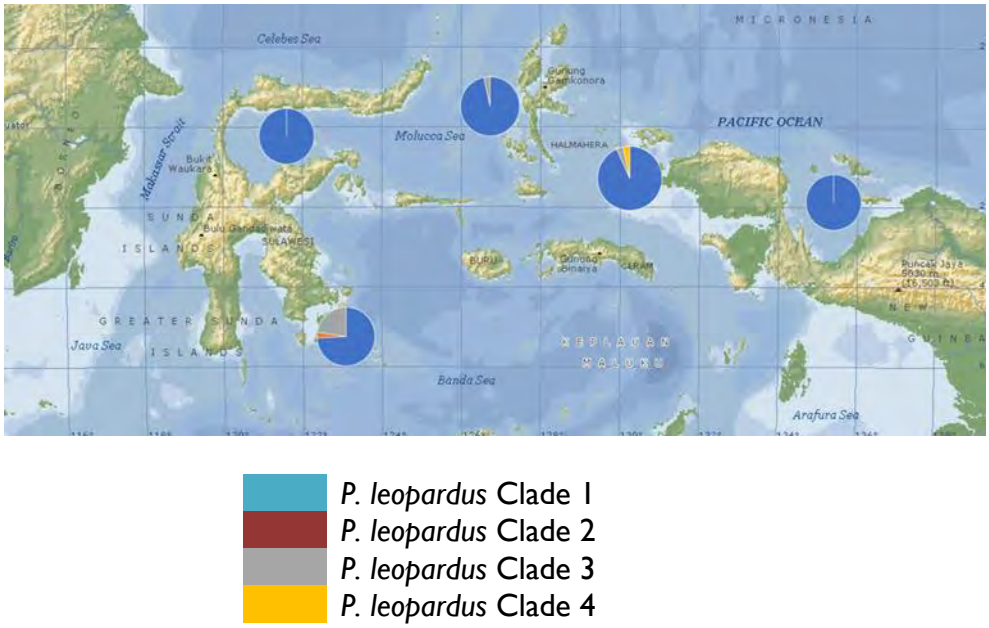

Figure 10. Overview of *P. leopardus* population structure at 5 sampling locations

3.4. DISCUSSION

Based on the criteria of genetic diversity, as categorized based on haplotype diversity, from Nei (1987) and referred by Akbar & Labenua (2018), there are three different levels of genetic diversity, namely low (0.1-0.4), moderate (0.5-0.7) and high (0.8-1.00). Based on the haplotype diversity of *L. malabaricus* as presented in Table 1, the level of genetic diversity of that species from Raja Ampat was categorized as moderate, while that from other four sampling location was categorized as high. On the other hand, as indicated by the haplotype diversity presented in Table 4, the level of genetic diversity *P. leopardus* from all sampling locations was high.

Avise et al. (1989) stated that the overall haplotype diversity of mtDNA for several fish was in the range 0.473-0.998. Nuryanto & Kochzius (2009) stated that high genetic diversity reflects a large population size, whereas a reduction in population size entails a reduction of genetic diversity. In their study, Nuryanto & Kochzius (2009) discussed that reduction of the genetic diversity of the giant clam (Tridacna maxima) was an impact of re-colonization, overexploitation and coral reef degradation. Referring to Nuryanto & Kochzius (2009), the high value of genetic diversity of *L. malabaricus* and *P. leopardus* may indicates that exploitation has not yet reached the overfishing stage, assuming that there was no habitat degradation. As a comparison, *L. malabaricus* from the Java has lower genetic diversity, which was around 0.6 - 0.76 (Soewardi & Suwarso, 2006). The Java Sea was a fishing ground with high fish stock exploitation level and degraded habitat impacted by the operation of demersal fishing gears.

AMOVA results showed that the fixation index (Fst) of *L. malabaricus* was categorized as moderate (0.4 - 0.7), while *P. leopardus* was categorized as low (0.1 - 0.3) (Excoffier et al., 1992). The lower Fst value shows the lower genetic variation between populations, this can be seen from the results of *P. leopardus*, which has a higher percentage of genetic variation within population than genetic variation among populations. Genetic variation in *L. malabaricus* has almost the same percentage between genetic variation within

population and genetic variation among population. The high and low percentage of genetic variation will affect the value of the gene flow. Gene flow values obtained by both *L. malabaricus* and *P. leopardus* were in the high category (Nm> 1), which indicates the low genetic difference between these two fish species at the sampling location. The difference in the percentage of genetic variation will affect the distribution of the haplotype group (clade). The dominance of one clade and present at all sampling locations indicates a low genetic difference. As obtained from this study, it can be seen that clade 1 is distributed in all sampling locations, even for *P. leopardus* is dominant in all sampling locations.

The result of analysis on the genetic distance (Tables 2 and 5) is in accordance with that of the Fst P Value (Tables 3 and 6). These indicate that the genetic distance of each fish species, i.e. *L. malabaricus* and *P. leopardus*, from the three sampling areas in FMA 715 locations are not significantly different. Either *L. malabaricus* or *P. leopardus* from the three sampling areas had a very close relationship. The closeness of this relationship could also be interpreted that each of that fish species from the three sampling locations came from the same population or in other words have same origins. The genetic distance value and the Fst P value also indicates that although each of *L. malabaricus* or *P. leopardus* in the three sampling locations came from the same population, the genetic relationship of those fish species in Ternate were closer to those in Raja Ampat, than those in Pagimana. This was presumably because geographically between Ternate and Pagimana are separated by deep waters, besides that Pagimana is located in the waters of Tomini Bay which tend to be more isolated than other waters. The existence of a gene flow caused by a current called the South Pacific Thermocline (Figure 11) was also one of the reasons for the sample from Ternate to be closer genetically to Raja Ampat.

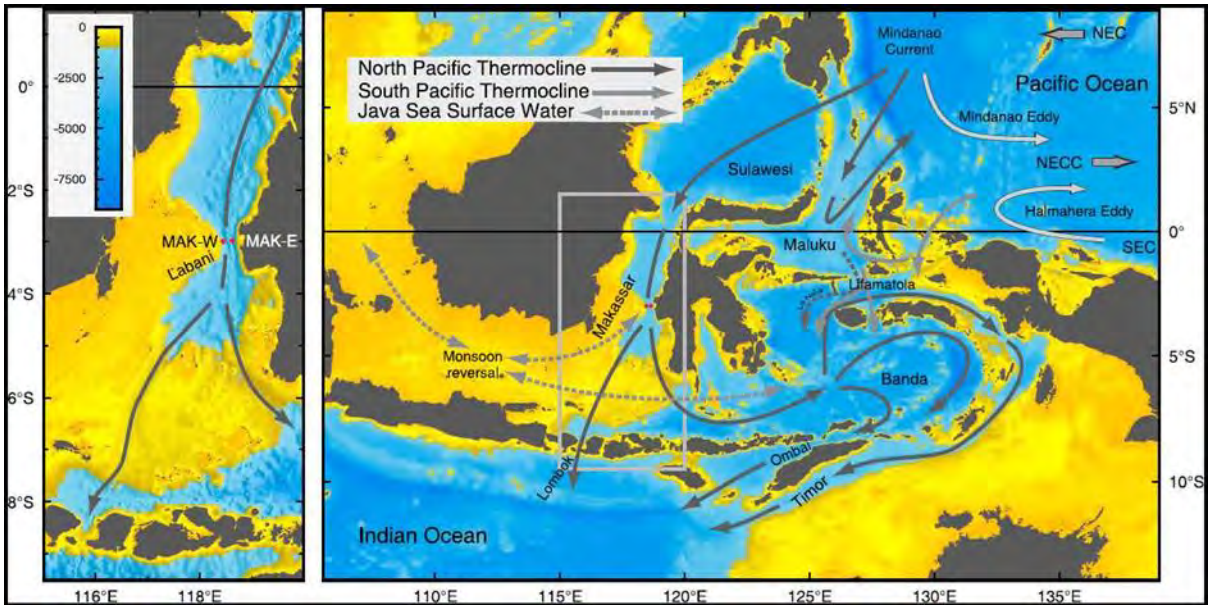

Figure 11. Water current system of Sulawesi and Maluku waters (Green et al., 2004)

However, the conclusion of this study that each of *L. malabaricus* and *P. leopardus* from the three sampling locations in FMA 715 came from the same population or in other words have same origins is based on the result of genetic analysis of mitochondrial DNA (mtDNA). The mtDNA is a genetic marker inherited from the maternal line only, so this is a potential difficulty in the analysis of stock structures (Antoniou & Magoulas, 2014). The conclusion of this study, using the mtDNA analysis, that each of *L. malabaricus* and

P. leopardus is not different among samples from the three sampling locations in FMA 715, cannot be used as a basis to say that *L. malabaricus* or *P. leopardus* in FMA 715 belong to the same stock.

Referring to NOAA (2006), stock is a part of a fish population usually with a particular migration pattern, specific spawning grounds, and subject to a distinct fishery. Based on the definition that the stock is an intraspecific group of randomly mating individuals with temporal and spatial integrity (Ihssen et al., 1981; Smith et al., 1990; Donovan, 1991), genetic characteristics that are inherited from male and female parents may not be identifiable from mtDNA analysis. In accordance with biochemical and genetic views, Cross & Payne (1978) define fish stocks as a group of individuals who have the same composition of allelomorphic genes, as a result of random mating in a location that was isolated from other populations. So that a species can have different allelomorphic genes based on geographic distribution.

The genetic information to decide the unit of stock can be obtained from genetic markers on the cell nucleus DNA, because it is derived from the line of father (male) and mother (female) and is unique to each individual (Schleif, 2004). Therefore, further research needs to be undertaken to analyze genetic markers on the cell nucleus DNA. One of the methods that is often used in cell nucleus DNA analysis is microsatellite. Microsatellites are very appropriate for the study of gene flow and mating systems, because microsatellite often shows wide variations (Finkeldey, 2005).

However, management of the fishery targeting *Lutjanus malabaricus* and *Plectropomus leopardus* in FMA 715 should be undertaken without waiting for complete scientific information. The effectiveness of fisheries management is, inter alia, affected by the ability of the government or management authority to control fishing intensity and regulate the anthropogenic risks facing fish stocks in a management unit (MU). A management unit defined without considering either the specific management objectives or the anthropogenic risks facing the populations can result in a management failure by losing local populations (Taylor & Dizon, 1999). Unfortunately, the question on whether *Lutjanus malabaricus* and *Plectropomus leopardus* in FMA 715 consisted of three stocks could not be answered before genetic markers on the cell nucleus DNA were analyzed. For the purposes of reef fishery management, the options would be whether treating all fishing grounds in FMA 715 as a single MU or three separated MUs.

Treating those fish species in three fishing areas as a single stock created risk of management failure if they actually belonged to different stocks, which have different growth, maturity, and mortality levels. Similar to the case discussed by Taylor & Dizon (1999), when three different fish stocks experienced different mortality levels, but were managed using the same management strategy, the optimal stock level would not be achieved, since the stocks with the highest and the lowest fishing pressures would be in suboptimal conditions. Three different stocks may require three different management strategies to effectively achieve optimal utilization.

Majority of species belong to families Lutjanidae and Serranidae live in sea waters shallower than 400 meters depth (Allen, 1985; Heemstra & Randall, 1993; fishbase.org). Based on bathymetric conditions of FMA 715, majority of the sea waters shallower than 400 meters is in the area less than 12 nautical mile from coastline, which is the area under management responsibility of provincial governments (Act No. 23 year 2014). Furthermore, the stocks of reef fishery in FMA 715 were harvested by small scale fishery operated by local fishers living in the village close to each fishing area. To effectively controlling fishing intensity, it would be more effective if the fishery was managed by relevant provincial governments. The provincial government should develop a management strategy for each relevant fish stock.

3.5. CONCLUSION

The conclusions that can be drawn from this study are:

a. Fish samples from Biak - Nabire were identified as Pinjalo pinjalo species.
b. Based on mt DNA analysis, each fish species, i.e. *L. malabaricus* and *P. leopardus*, from the three sampling areas in FMA 715 locations are genetically not significantly different and kinship between Ternate and Raja Ampat was closer than between Ternate and Pagimana.
c. In Malabar blood snapper there are four clades, clade 1 is found in all populations and is dominant in FMA 715 waters (Pagimana, Ternate, Raja Ampat), 4 clades are also found in Leopard coral grouper fish with one of the dominant clades in all sampling locations.
d. The result of this study using the mtDNA analysis cannot be used as a basis to say that *L. malabaricus* or *P. leopardus* in FMA 715 belong to the same stock. Further research needs to be undertaken to analyze genetic markers on the cell nucleus DNA.

The management of the fishery targeting *Lutjanus malabaricus* and *Plectropomus leopardus* in FMA 715 should be carried out without waiting for complete scientific information. To effectively controlling fishing intensity, each provincial government should manage the fishery in its fisheries management area

4. MORPHOMETRIC CHARACTERISTICS

4.1. INTRODUCTION

Stock identification is a part of fisheries science that can support the fish stock assessment by defining the unit of fish stock to be assessed as a basis for fisheries management. The identification of stock can be carried out based on genetic variations and morphological or phenotypical characteristics. The delineation of fish stock units phenotypically is conducted by determining and comparing morphometric differences of fish from various fishing areas (Cadrin, 2000; Turan, 2004; Mekkawy & Mohammad, 2011).

Morphometrics are morphological characters of fishes that are used in the determination of the stock unit undertaken based on fish body shape parameters (Turan, 2004). Many individual characteristics are phenotypically plastic whereby a single genotype can develop different phenotypes in different environments (Hard, 1995; Sultan & Stearns, 2005). Morphometric characters in defining unit stock are often used and not sensitive to changes in short-term, local fluctuations and reflects the areas difference in average environmental factors over a long period (Ismen, 2001).

Morphological or phenotypic observations to identify fish stocks and define stock structures have been widely applied for demersal and reef fish groups. Several previous studies have been carried out to identify the stock of reef fish species using the morphometric approach, for example for snapper *Lutjanus quinquelineatus* (Soliman et al, 2018), and grouper *Plectropomus pessuliferus*, and *P. areolatus* (Elamin et al, 2011). This method is also used for other demersal species, for instance *Lates calcarifer* (Gopikrishna et al., 2006), *Harpodon nehereus* (Pazhayamadom et al., 2014), *Sillaginopsis panijus* (Siddik et al., 2016) and *Eubleekeria splendens* (Rawat et al., 2018).

Malabar blood snapper (*Lutjanus malabaricus*) and Leopard coral grouper (*Plectropomus leopardus*) inhabit in Fisheries Management Area (FMA) 715 of Indonesia. The area is crossed by Indonesian Throughflow eastern pathways, which brings water masses from Western North Pacific Ocean and exits to Indian Ocean (Ffield & Gordon, 1992). FMA 715 covers many islands and various bathymetric characteristics. Significant impact of different physical characteristics to phenotype characters of reef fish species is found by Bay et al. (2008). Bay et al. (2008) reveals the existence of the three fish stocks of *Acanthochromis polyacanthus* in the Great Barrier Reef as significant impact of distance amongst the three regions.

Considering possible the impact of the distance among areas to phenotype characters of species *P. leopardus* and *L. malabaricus* in the areas of FMAs 714, 715 and 716, this study aims at identifying the stock structure and stock delineation of those two reef fish species in those three FMAs. The result of this study would complement the genetic study of the same species presented in this report.

4.2. METHODS

Morphological or phenotypical characteristics of *P. leopardus* and *L. malabaricus* were analysed by using data of eighteen morphometric characters. The morphometric data were collected by measuring each sample of those two species collected from the fishers. digital caliper was used in the measurement. As was done by Soliman et al. (2018), the morphometrics characters that were measured in this study (Figure 12) are:

(1) Total length (TL)
(2) Standard length (TL)
(3) Body depth (DB)
(4) Caudal peduncle depth (CPD)
(5) Head length (HL)
(6) Predorsal fin length (PRDFL)
(7) Head depth (HD)
(8) Preventral fin length (PRDFL)
(9) Distance between ventral and dorsal fins origin (VDOL)
(10) Distance between anal and dorsal fin ends (ADFEL)
(11) Dorsal fin base length (DFBL)
(12) Distance between the ventral fin origin and the end of anal fin (VOAEFL)
(13) Distance between the first spine of the dorsal fin and the end of anal fin (SPDAEFL)
(14) Distance between dorsal fin end and ventral fin origin (DEVOFL)
(15) Distance between the ventral fin and the end of fin origin (VEADFL)
(16) Distance between dorsal fin end and dorsal caudal fin origin (DEDCF)
(17) Distance between anal fin end and ventral caudal fin origin (AEVCFL)
(18) Eye diameter (ED).

The analysis was carried out on 18 morphometric characters of Malabar blood snapper from seven location and Leopard coral grouper from five locations. The Kruskal-Wallis test, cluster analysis and discriminant analysis were performed to identifying the group population among sampling locations. The Kruskal-Wallis test was used to trace the significant difference of morphometric characters among the fish in all sampling locations (Ostertagova et al. 2014). Cluster analysis was used to decide the similarity between which formed based on morphometric characters. Discriminant analysis was conducted to determine population grouping based on the different locations (Hidayani et al. 2015; Zairion et al. 2020).

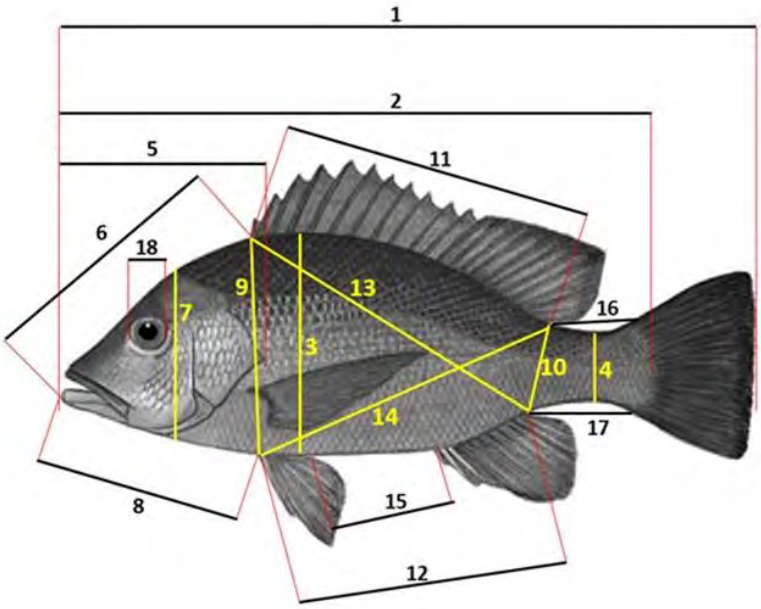

Figure 12. Morphometric measurement which applied for *Lutjanus malabaricus* and *Plectropomus leopardus* from eastern part of Indonesian waters

4.3. RESULT

4.3.1. MALABAR BLOOD SNAPPER (*Lutjanus malabaricus*)

a. Length and weight measurement

The total number of Malabar blood snapper collected from seven sampling locations which was assumed as representation of its surrounding waters were 283 fishes which consisted of 137 males, 125 females and 21 unsexed. The mean and standard deviation of standard length and weight of Malabar blood snapper showed variation in seven sampling locations (Table 7). In general, it is known that the largest mean of standard length of this species was found in Kendari, while the smallest was in Raja Ampat. Moreover, the largest mean of weight of Malabar blood snapper was found in Tarakan and the smallest was in Raja Ampat.

b. Variation of morphometric characters

The value which was used in the multivariate analysis was the ratio of each morphometric characters to the standard length. The mean ratio of each morphometric characters in seven sampling locations was presented in Table 8, Table 9, and Table 10 for males, females, and pooled-sex, respectively. Furthermore, Kruskal-Wallis test was applied to identifying the significant difference of morphometric characters on each location. The Kruskal-Wallis test showed the variation in morphometric characters of Malabar blood snapper in all sampling locations, which was significantly different ($p < 0.05$) in all characters for both males, females, and pooled-sex (Table 8, Table 9 and Table 10). Thus, it is known that all morphometric characters were significantly contribute to the population grouping on seven sampling locations. It means that all of those characters should be included for further multivariate analysis such as cluster analysis and discriminant analysis.

Table 7. Length and weight of *Lutjanus malabaricus* collected in eastern part of Indonesian waters

Sex	Location	n	Standard length (cm)				Weight (g)			
			Min	Max	Mean	SD	Min	Max	Mean	SD
Male	Kendari	9	37.20	46.80	42.13	2.90	1078.00	2354.00	1702.00	411.36
	Bau-bau	21	22.20	38.20	26.40	3.41	331.00	1386.00	539.52	218.94
	Pagimana	21	26.30	43.20	34.78	4.74	511.00	2063.00	1151.48	431.05
	Ternate	18	25.30	54.50	39.08	7.14	476.60	3745.00	1630.51	795.27
	Raja Ampat	25	17.10	40.70	21.93	6.14	142.00	1668.00	367.52	409.10
	Tarakan	22	24.40	49.10	40.37	6.53	462.00	2904.00	1779.91	678.00
	BiakNabire	21	16.90	33.20	23.05	5.42	155.00	1146.00	425.75	320.47
Female	Kendari	5	36.60	43.20	40.50	2.80	1150.00	1751.00	1537.20	251.38
	Bau-bau	8	24.10	31.50	27.29	2.39	274.00	870.00	555.38	183.32
	Pagimana	10	25.50	48.70	38.55	7.33	458.00	2835.00	1647.60	793.58
	Ternate	32	23.60	58.50	33.62	6.77	419.10	5454.40	1334.32	1081.87
	Raja Ampat	25	15.30	30.50	19.98	2.90	115.00	915.00	244.36	150.17
	Tarakan	24	33.20	50.10	40.26	4.19	1072.00	3136.00	1739.21	477.33
	BiakNabire	21	17.00	32.50	21.70	4.38	146.00	1017.00	336.52	223.93
Pooled	Kendari	14	36.60	46.80	41.55	2.87	1078.00	2354.00	1643.14	360.96
	Bau-bau	30	21.70	38.20	26.48	3.22	274.00	1386.00	535.67	207.97
	Pagimana	31	25.50	48.70	36.00	5.86	458.00	2835.00	1311.52	606.94
	Ternate	50	23.60	58.50	35.58	7.33	419.10	5454.40	1440.95	990.22
	Raja Ampat	54	15.30	40.70	20.81	4.74	114.00	1668.00	296.19	301.67
	Tarakan	50	24.40	50.10	40.23	5.21	462.00	3136.00	1729.72	565.92
	BiakNabire	54	16.90	33.20	21.76	4.50	146.00	1146.00	348.70	249.10

Table 8. Morphometric characters comparison of male sex *L. malabaricus* in eastern part of Indonesian waters

Morphometric characters	Mean ratio							p-value
	Kendari	Bau-bau	Pagimana	Ternate	Raja Ampat	Tarakan	Biak-Nabire	
TL/SL	1.170	1.210	1.250	1.200	1.250	1.200	1.220	0.000
BD/SL	3.700	4.060	3.790	3.830	4.010	3.840	4.180	0.000
CPD/SL	1.120	1.200	1.050	1.170	1.180	1.140	1.230	0.000
HL/SL	3.380	3.580	3.540	3.540	3.880	3.480	3.980	0.000
PRDFL/SL	3.800	4.120	3.960	4.210	4.310	4.090	4.300	0.000
HD/SL	2.790	2.740	2.780	2.920	3.010	2.900	3.210	0.000
PRvDFL/SL	3.500	3.610	3.680	3.700	3.940	3.650	3.970	0.000
VDOL/SL	3.460	3.720	3.550	3.650	3.750	3.720	3.870	0.000
ADFEL/SL	1.380	1.490	1.350	1.550	1.470	1.500	1.600	0.000
DFBL/SL	4.780	5.050	5.000	5.130	5.130	5.040	5.230	0.000
VOAEFL/SL	4.270	4.300	4.230	4.400	4.480	4.410	4.470	0.000
SPDAEFL/SL	4.950	5.490	5.310	5.470	5.620	5.400	5.630	0.000
DEVOFL/SL	4.990	5.060	4.970	5.140	5.190	4.970	5.250	0.000
VEADFL/SL	2.420	2.470	2.420	2.500	2.640	2.390	2.620	0.000
DEDCF/SL	1.720	1.750	1.590	1.670	1.610	1.520	1.590	0.000
AEVCFL/SL	1.960	1.890	1.750	1.880	1.810	1.740	1.860	0.000
ED/SL	0.616	0.754	0.598	0.667	0.894	0.607	1.020	0.000
N	**9**	**21**	**21**	**18**	**25**	**22**	**21**	

Table 9. Morphometric characters comparison of female *L. malabaricus* in eastern part of Indonesian waters

Morphometric characters	Mean ratio							p-value
	Kendari	Bau-bau	Pagimana	Ternate	Raja Ampat	Tarakan	Biak-Nabire	
TL/SL	1.200	1.210	1.230	1.200	1.230	1.190	1.210	0.000
BD/SL	3.770	4.040	3.790	3.900	3.950	3.800	4.020	0.000
CPD/SL	1.120	1.210	1.050	1.180	1.190	1.150	1.210	0.000
HL/SL	3.420	3.620	3.590	3.800	3.870	3.610	3.900	0.000
PRDFL/SL	3.900	4.130	3.970	4.140	4.370	4.090	4.230	0.000
HD/SL	2.760	2.800	2.690	3.060	2.930	2.880	3.140	0.000
PRvDFL/SL	3.570	3.730	3.660	3.870	3.990	3.660	3.880	0.000
VDOL/SL	3.480	3.770	3.550	3.790	3.750	3.660	3.910	0.000
ADFEL/SL	1.450	1.500	1.380	1.570	1.500	1.490	1.690	0.000
DFBL/SL	4.460	4.970	4.960	5.250	5.080	5.050	5.170	0.000
VOAEFL/SL	4.340	4.330	4.270	4.370	4.470	4.410	4.440	0.003
SPDAEFL/SL	5.230	5.470	5.370	5.700	5.570	5.330	5.520	0.000
DEVOFL/SL	5.000	5.070	5.020	5.140	5.150	5.160	5.170	0.003
VEADFL/SL	2.490	2.440	2.430	2.580	2.620	2.440	2.600	0.000
DEDCF/SL	1.820	1.630	1.620	1.610	1.620	1.490	1.650	0.000
AEVCFL/SL	2.010	1.890	1.800	1.880	1.800	1.740	1.860	0.000
ED/SL	0.628	0.760	0.595	0.772	0.932	0.613	1.030	0.000
N	**5**	**8**	**10**	**32**	**25**	**24**	**21**	

Table 10. Morphometric characters comparison of pooled *L. malabaricus* in eastern part of Indonesian waters

Morphometric charaters	Mean ratio							p-value
	Kendari	Bau-bau	Pagimana	Ternate	Raja Ampat	Tarakan	Biak-Nabire	
TL/SL	1.182	1.213	1.240	1.199	1.240	1.193	1.218	0.000
BD/SL	3.721	4.063	3.788	3.877	3.980	3.803	4.107	0.000
CPD/SL	1.120	1.208	1.052	1.177	1.183	1.146	1.233	0.000
HL/SL	3.396	3.592	3.555	3.708	3.869	3.555	3.922	0.000
PRDFL/SL	3.835	4.129	3.961	4.165	4.337	4.101	4.308	0.000
HD/SL	2.777	2.759	2.751	3.009	2.965	2.882	3.218	0.000
PRvDFL/SL	3.526	3.642	3.672	3.806	3.963	3.657	3.937	0.000
VDOL/SL	3.466	3.735	3.548	3.742	3.742	3.678	3.913	0.000
ADFEL/SL	1.405	1.498	1.361	1.566	1.475	1.494	1.645	0.000
DFBL/SL	4.668	5.033	4.988	5.204	5.100	5.039	5.213	0.000
VOAEFL/SL	4.292	4.304	4.242	4.379	4.474	4.414	4.479	0.000
SPDAEFL/SL	5.052	5.477	5.330	5.616	5.588	5.364	5.587	0.000
DEVOFL/SL	4.997	5.078	4.989	5.137	5.167	5.080	5.231	0.000
VEADFL/SL	2.448	2.459	2.422	2.553	2.622	2.405	2.608	0.000
DEDCF/SL	1.754	1.716	1.597	1.629	1.612	1.499	1.581	0.000
AEVCFL/SL	1.982	1.892	1.768	1.880	1.800	1.735	1.823	0.000
ED/SL	0.621	0.757	0.597	0.734	0.913	0.609	1.035	0.000
N	**14**	**30**	**31**	**50**	**54**	**50**	**54**	

c. Population proximity grouping

The logarithm transformation of the ratio value of morphometric characters was conducted before the multivariate (cluster and discriminant) analysis. The result of cluster analysis in all sexes from all sampling location tend to have the same pattern, where there were two main clusters of Malabar blood snapper population. In the first main branch, there were two populations, that are Raja Ampat dan Biak-Nabire, while the second main branch consisted of five populations (Kendari, Bau-bau, Pagimana, Ternate and Tarakan) as shown in the Figure 13. Form this graph, we can see that Malabar blood snapper from Raja Ampat and Biak-Nabire were highly separated from five other locations, and this pattern was occurred in all sexes. In males and females, they also present that Kendari population was separated from four others location (Bau-bau, Pagimana, Ternate, Tarakan) in the second main cluster. Generally, it is known that Malabar blood snapper in this study had three distinct population, viz.: 1) Raja Ampat and Biak-Nabire; 2) Kendari and Pagimana; and 3) Bau-bau, Ternate, and Tarakan.

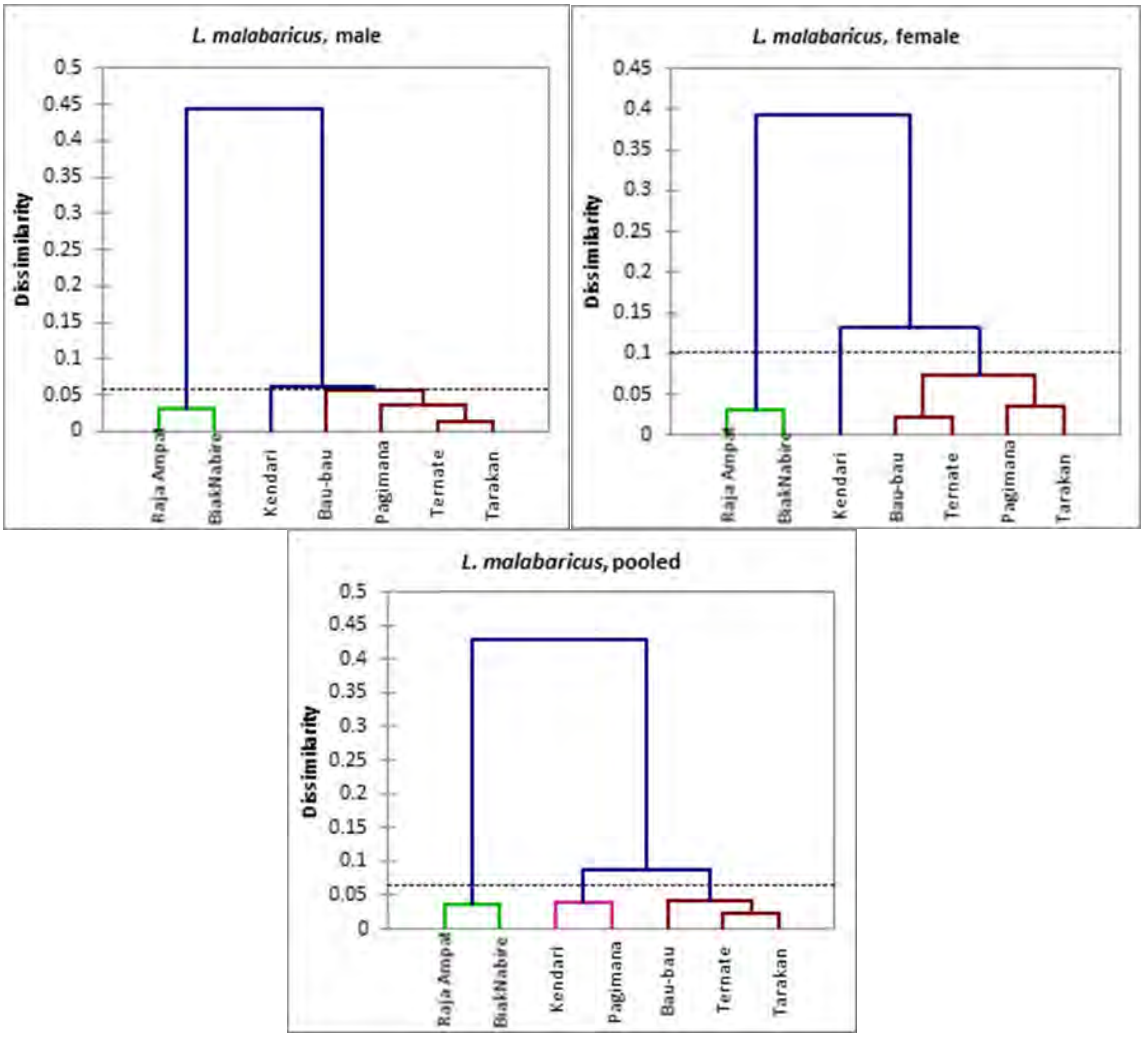

Figure 13. The result of cluster analysis of the *L. malabaricus* morphometric characters in eastern part of Indonesia waters

Population proximity grouping of Malabar blood snapper in cluster analysis could also be seen based on the value of Euclidean distance as presented in the Table 11, the greater Euclidean distance value indicated

that the similarity among groups getting smaller, on the contrary the smaller distance value presents the bigger similarity among groups. For example, in pooled sexes Malabar blood snapper, the population from Ternate were closer to Tarakan than the other locations where the value of Euclidean distance was 0.217 which smaller than others distance.

Table 11. Euclidean distance of male, female and pooled-sex of *L. malabaricus* in eastern part of Indonesia waters

Sex	Euclidean distance						
	Kendari	**Bau-bau**	**Pagimana**	**Ternate**	**Raja Ampat**	**Tarakan**	**BiakNabire**
Male							
Kendari	0.000	0.352	0.283	0.274	0.621	0.366	0.792
Bau-bau	0.352	0.000	0.319	0.248	0.396	0.336	0.560
Pagimana	0.283	0.319	0.000	0.243	0.504	0.256	0.705
Ternate	0.274	0.248	0.243	0.000	0.483	0.172	0.641
Raja Ampat	0.621	0.396	0.504	0.483	0.000	0.519	0.246
Tarakan	0.366	0.336	0.256	0.172	0.519	0.000	0.670
BiakNabire	0.792	0.560	0.705	0.641	0.246	0.670	0.000
Female							
Kendari	0.000	0.467	0.307	0.523	0.728	0.449	0.839
Bau-bau	0.467	0.000	0.327	0.209	0.366	0.288	0.470
Pagimana	0.307	0.327	0.000	0.370	0.583	0.263	0.718
Ternate	0.523	0.209	0.370	0.000	0.344	0.285	0.401
Raja Ampat	0.728	0.366	0.583	0.344	0.000	0.541	0.248
Tarakan	0.449	0.288	0.263	0.285	0.541	0.000	0.637
BiakNabire	0.839	0.470	0.718	0.401	0.248	0.637	0.000
Pooled							
Kendari	0.000	0.370	0.277	0.388	0.663	0.380	0.839
Bau-bau	0.370	0.000	0.311	0.221	0.394	0.316	0.559
Pagimana	0.277	0.311	0.000	0.295	0.543	0.252	0.738
Ternate	0.388	0.221	0.295	0.000	0.381	0.217	0.512
Raja Ampat	0.663	0.394	0.543	0.381	0.000	0.525	0.268
Tarakan	0.380	0.316	0.252	0.217	0.525	0.000	0.658
BiakNabire	0.839	0.559	0.738	0.512	0.268	0.658	0.000

In addition to the cluster analysis, the proximity population grouping could also be analyzed using discriminant analysis. The discriminant analysis presents plot distribution of morphometric characters from all sampling locations as well as its centroid. Figure 14 indicates that the population of Malabar blood snapper from Tarakan, Ternate, Kendari and Bau-bau are not separated, on the other hand the population from Pagimana, Biak-Nabire and Raja Ampat were indicated as different group of population. This condition showed clearly in the male and pooled sexes. Moreover, if we only look at the location in the Fisheries Management Area (FMA) 715 (Pagimana, Ternate, Raja Ampat), it is known that the population of Malabar blood snapper from these three locations were separated each other.

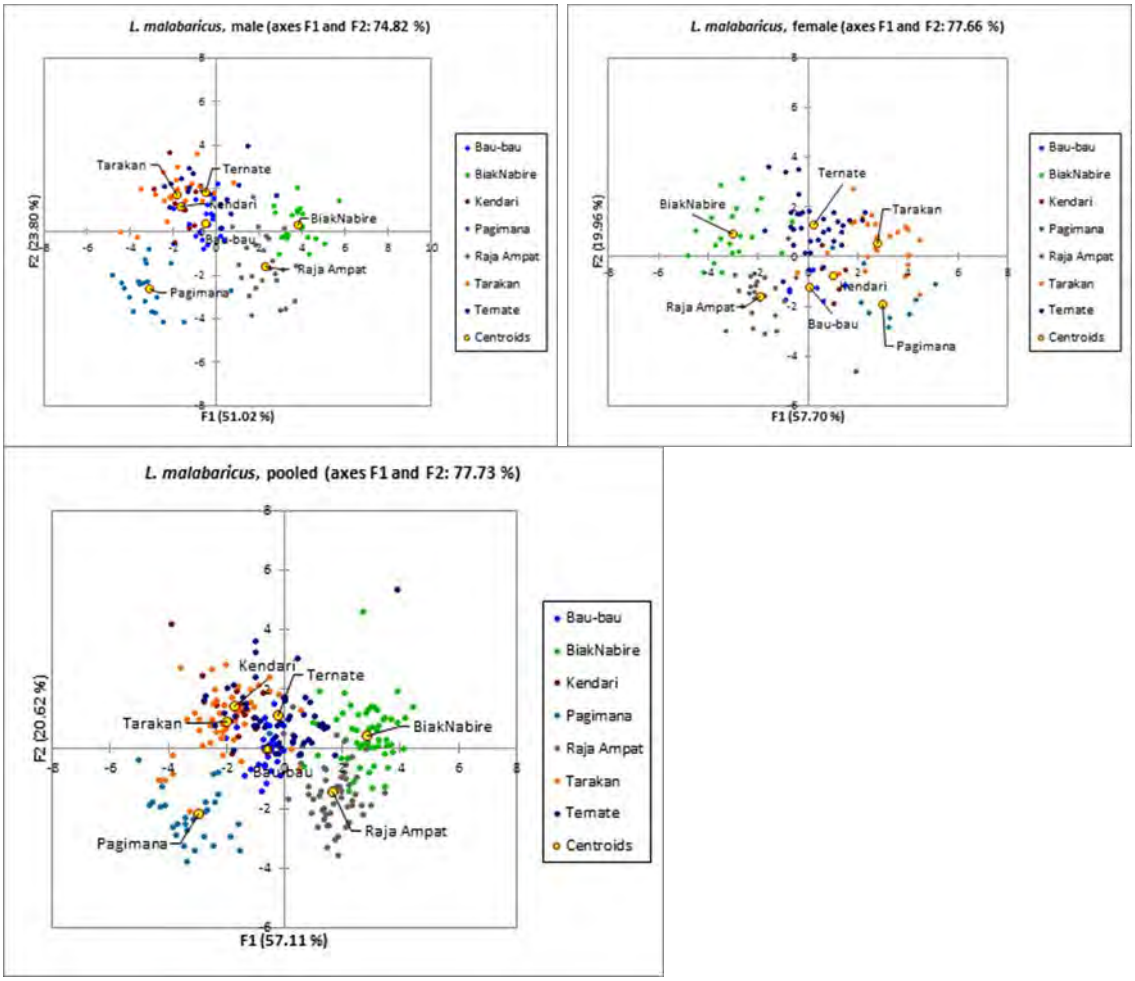

Figure 14. Morphometric characters distribution plot of *L. malabaricus* in seven locations in eastern part of Indonesia waters

According to the group prediction, it is known that some fishes point the similarity to others location. This phenomenon provides in the Table 12, where one fish of males Malabar blood snapper population from Kendari belong to the fish population of Bau-bau. One fish from Bau-bau has the similarity to the fishes from Ternate. Five fishes from Ternate belongs to the population of Tarakan and one fish belong to the Biak-Nabire population. Group prediction based on the morphometric characters from the others locations present in the Table 12.

Table 12. Group prediction among sex of *L. malabaricus* in sampling location

Sex	Location	Kendari	Bau-bau	Pagimana	Ternate	Raja Ampat	Tarakan	Biak-Nabire	Total	% correct
Male	Kendari	8	1	0	0	0	0	0	9	88.89
	Bau-bau	0	20	0	1	0	0	0	21	95.24
	Pagimana	0	0	20	0	1	0	0	21	95.24
	Ternate	0	0	0	12	0	5	1	18	66.67
	Raja Ampat	0	0	0	2	22	0	1	25	88.00
	Tarakan	0	0	0	4	0	18	0	22	81.82
	BiakNabire	0	0	0	0	0	0	21	21	100.00
	Over-all	8	21	20	19	23	23	23	137	87.98
Female	Kendari	4	1	0	0	0	0	0	5	80.00
	Bau-bau	0	8	0	0	0	0	0	8	100.00
	Pagimana	0	0	9	0	0	1	0	10	90.00
	Ternate	0	1	0	28	1	2	0	32	87.50
	Raja Ampat	0	0	0	1	24	0	0	25	96.00
	Tarakan	0	0	1	2	1	20	0	24	83.33
	BiakNabire	0	0	0	1	1	0	19	21	90.48
	Over-all	4	10	10	32	27	23	19	125	89.62
Pooled	Kendari	10	2	0	2	0	0	0	14	71.43
	Bau-bau	0	27	0	2	1	0	0	30	90.00
	Pagimana	0	2	27	0	1	1	0	31	87.10
	Ternate	1	2	0	33	0	10	4	50	66.00
	Raja Ampat	0	0	0	4	46	0	4	54	85.19
	Tarakan	1	0	4	6	1	38	0	50	76.00
	BiakNabire	0	0	0	3	7	0	44	54	81.48
	Over-all	12	33	31	50	56	49	52	283	79.60

4.3.2. LEOPARD CORAL GROUPPER (*Plectropomus leopardus*)

a. Length and weight measurement

In contrast to the Malabar blood snapper, the Leopard coral grouper only found in five sampling locations. The total of Leopard coral grouper from five sampling locations were 227 fishes, which consisted of 71 males, 152 females and 4 fishes unsexed. The mean and standard deviation of standard length and weight of Leopard coral grouper showed the variation (Table 13). Generally, the largest mean of standard length of this species was found in Raja Ampat, while the smallest mean in Wakatobi. Meanwhile, the largest mean of weight of this fish was found in Ternate and the smallest mean weight in Wakatobi.

b. Variation of morphometrics characters

The value which were used in the multivariate analysis was the ratio of each morphometric characters to the standard length. The mean ratio of each morphometric characters in five sampling locations was presented in Table 14, Table 15, and Table 16 for males, females, and pooled sex, respectively. Furthermore, Kruskal-Wallis test was adjusted to identifying the significant difference of morphometric characters on each location. The Kruskal-Wallis test presents the variation in morphometric characters of Leopard coral grouper in all sampling locations, which was significantly different ($p < 0.05$) in all characters for both males, females, and pooled-sex (Table 14, Table 15, and Table 16). Thus, it is known that all morphometric characters were significantly contribute to the population grouping on five sampling locations. It means that all of those characters should be included for further multivariate analysis such as cluster analysis and discriminant analysis.

Table 13. Length and weight structure of *Plectropomus leopardus* collected in eastern part of Indonesian waters

Sex/Location		n	Standard length (cm)				Weight (g)			
			Min	Max	Mean	SD	Min	Max	Mean	SD
Male	Wakatobi	19	21.50	27.30	25.01	1.89	233.00	503.00	381.21	84.63
	Pagimana	10	22.90	34.20	28.92	4.24	304.00	1014.00	666.90	288.11
	Raja Ampat	8	27.50	41.30	34.84	5.14	593.00	1701.00	1082.75	417.47
	Biak-Nabire	22	23.60	41.60	29.97	5.10	278.00	2155.00	722.73	454.56
	Ternate	12	19.10	43.00	32.73	7.08	181.40	2000.00	945.68	569.67
Female	Wakatobi	32	18.70	37.40	24.58	4.12	146.00	1412.00	383.63	254.15
	Pagimana	29	17.60	41.70	27.28	6.14	117.00	1593.00	565.10	371.45
	Raja Ampat	25	22.50	43.80	32.52	5.12	302.00	1996.00	893.24	428.66
	Biak-Nabire	29	19.30	36.50	26.31	4.78	166.00	1096.00	468.38	268.61
	Ternate	37	21.30	42.00	32.86	5.76	231.60	2077.00	981.84	496.54
Pooled	Wakatobi	52	18.70	37.40	24.73	3.41	146.00	1412.00	381.92	204.51
	Pagimana	39	17.60	41.70	27.70	5.71	117.00	1593.00	591.21	351.22
	Raja Ampat	33	22.50	43.80	33.08	5.15	302.00	1996.00	939.18	427.48
	Biak-Nabire	53	15.60	41.60	27.55	5.43	94.00	2155.00	562.49	379.97
	Ternate	50	19.10	43.00	32.73	6.01	181.40	2077.00	965.58	506.93

Table 14. Morphometric characters comparison of male sex *P. leopardus* in eastern part of Indonesian waters

Morphometric characters	**Mean Ratio**					**p-value**
	Wakatobi	**Pagimana**	**Raja Ampat**	**BiakNabire**	**Ternate**	
TL/SL	0.09	0.08	0.07	0.08	0.07	0.000
BD/SL	0.45	0.49	0.46	0.48	0.45	0.000
CPD/SL	0.10	0.12	0.12	0.10	0.11	0.000
HL/SL	0.53	0.54	0.53	0.53	0.52	0.000
PRDFL/SL	0.59	0.61	0.60	0.59	0.59	0.000
HD/SL	0.35	0.33	0.36	0.34	0.32	0.000
PRvDFL/SL	0.52	0.53	0.52	0.52	0.51	0.000
VDOL/SL	0.44	0.46	0.45	0.44	0.45	0.000
ADFEL/SL	0.19	0.23	0.22	0.19	0.22	0.000
DFBL/SL	0.66	0.66	0.65	0.64	0.64	0.000
VOAEFL/SL	0.69	0.70	0.68	0.68	0.69	0.000
SPDAEFL/SL	0.69	0.67	0.69	0.68	0.69	0.000
DEVOFL/SL	0.73	0.74	0.73	0.73	0.73	0.000
VEADFL/SL	0.46	0.50	0.49	0.47	0.50	0.000
DEDCF/SL	0.27	0.27	0.27	0.30	0.27	0.000
AEVCFL/SL	0.32	0.31	0.28	0.31	0.28	0.000
ED/SL	-0.20	-0.23	-0.25	-0.22	-0.28	0.000
n	19	10	8	22	12	

Table 15. Morphometric characters comparison of female *P. leopardus* in eastern part of Indonesian waters

Morphometric characters	Mean Ratio					p-value
	Wakatobi	Pagimana	Raja Ampat	BiakNabire	Ternate	
TL/SL	0.08	0.07	0.08	0.08	0.08	0.000
BD/SL	0.45	0.46	0.48	0.45	0.46	0.000
CPD/SL	0.10	0.11	0.12	0.07	0.11	0.000
HL/SL	0.53	0.53	0.53	0.53	0.53	0.000
PRDFL/SL	0.59	0.62	0.60	0.59	0.60	0.000
HD/SL	0.33	0.34	0.37	0.32	0.33	0.000
PRvDFL/SL	0.52	0.54	0.52	0.51	0.52	0.000
VDOL/SL	0.44	0.45	0.45	0.42	0.46	0.000
ADFEL/SL	0.20	0.20	0.21	0.17	0.22	0.000
DFBL/SL	0.65	0.65	0.65	0.64	0.65	0.000
VOAEFL/SL	0.69	0.69	0.68	0.68	0.69	0.000
SPDAEFL/SL	0.69	0.69	0.68	0.68	0.69	0.000
DEVOFL/SL	0.73	0.72	0.72	0.72	0.73	0.000
VEADFL/SL	0.47	0.49	0.51	0.46	0.49	0.000
DEDCF/SL	0.28	0.24	0.30	0.28	0.29	0.000
AEVCFL/SL	0.32	0.26	0.30	0.30	0.30	0.000
ED/SL	-0.21	-0.19	-0.23	-0.23	-0.26	0.000
n	32	29	25	29	37	

Table 16. Morphometric characters comparison of pooled *P. leopardus* in eastern part of Indonesian waters

Morphometric characters	Mean Ratio					p-value
	Wakatobi	Pagimana	Raja Ampat	BiakNabire	Ternate	
TL/SL	0.08	0.08	0.08	0.08	0.07	0.000
BD/SL	0.45	0.47	0.47	0.47	0.46	0.000
CPD/SL	0.10	0.11	0.12	0.08	0.11	0.000
HL/SL	0.53	0.53	0.53	0.53	0.53	0.000
PRDFL/SL	0.59	0.62	0.60	0.59	0.60	0.000
HD/SL	0.34	0.34	0.37	0.33	0.33	0.000
PRvDFL/SL	0.52	0.53	0.52	0.52	0.51	0.000
VDOL/SL	0.44	0.45	0.45	0.42	0.46	0.000
ADFEL/SL	0.20	0.21	0.21	0.18	0.22	0.000
DFBL/SL	0.65	0.65	0.65	0.64	0.65	0.000
VOAEFL/SL	0.69	0.69	0.68	0.68	0.69	0.000
SPDAEFL/SL	0.69	0.69	0.68	0.68	0.69	0.000
DEVOFL/SL	0.73	0.73	0.72	0.72	0.73	0.000
VEADFL/SL	0.47	0.49	0.50	0.46	0.49	0.000
DEDCF/SL	0.28	0.25	0.29	0.29	0.28	0.000
AEVCFL/SL	0.32	0.28	0.30	0.30	0.30	0.000
ED/SL	-0.21	-0.20	-0.23	-0.23	-0.27	0.000
n	52	39	33	53	50	

c. Population proximity grouping

As well as the analysis of Malabar blood snapper, the logarithm transformation of the ratio value of morphometric characters was also conducted in Leopard coral grouper before the multivariate (cluster and discriminant) analysis. The result of cluster analysis in all sexes from all sampling location tend to have the same pattern, where there were two main clusters of Leopard coral grouper population. In the first main branch, there were two populations (Wakatobi and Biak-Nabire) and the second main branch consisted of three populations (Pagimana, Ternate and Raja Ampat) as shown in the Figure 15. Form this graph, we can see that Leopard coral grouper from Wakatobi and Biak-Nabire were highly separated from three others locations, and this pattern showed a difference in females, where the highly separated population found on Pagimana. Generally, it is known that Leopard coral grouper in this study had three different population, that are: 1) Wakatobi and Biak-Nabire; 2) Pagimana; and 3) Raja Ampat and Ternate.

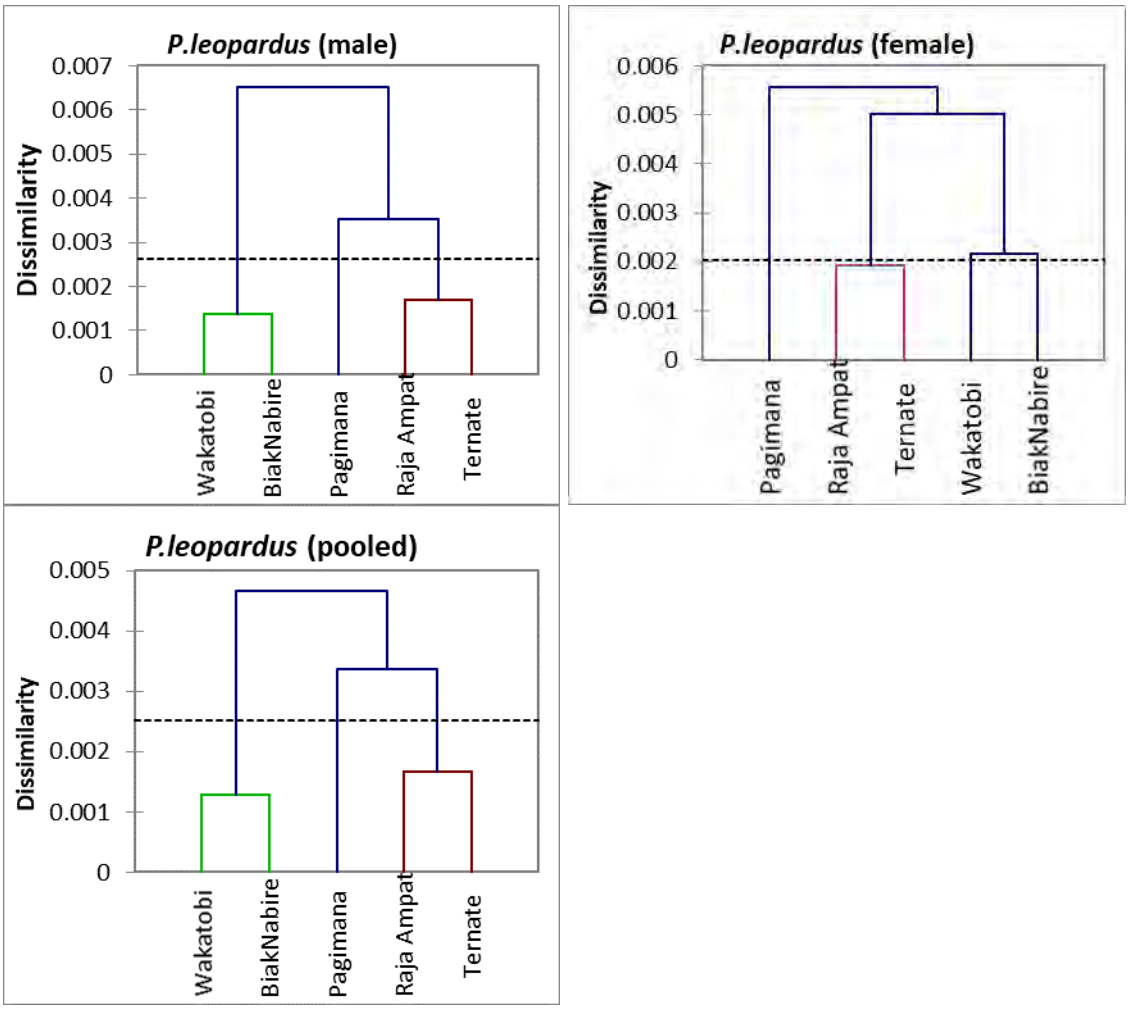

Figure 15. The result of cluster analysis of the *P. leopardus* morphometric characters in eastern part of Indonesia waters

Population proximity grouping of Leopard coral grouper in cluster analysis could also be seen based on the value of Euclidean distance as presented in the Table 17, the greater Euclidean distance value indicated that the similarity among groups getting smaller and in contrast the smaller distance value presents the

bigger similarity among groups. For example, in males Leopard coral grouper, where the distance of Ternate population was closer to the Pagimana (0.086) and Raja Ampat (0.058) compared to Wakatobi (0.107) and Biak-Nabire (0.103) population.

Table 17. Euclidean distance of male, female and pooled sex of *P. leopardus* in eastern part of Indonesia waters

Sex	Location	Euclidean distance				
		Wakatobi	Pagimana	Raja Ampat	BiakNabire	Ternate
Male	Wakatobi	0.000	0.080	0.082	0.052	0.107
	Pagimana	0.080	0.000	0.070	0.076	0.086
	Raja Ampat	0.082	0.070	0.000	0.080	0.058
	BiakNabire	0.052	0.076	0.080	0.000	0.103
	Ternate	0.107	0.086	0.058	0.103	0.000
Female	Wakatobi	0.000	0.086	0.066	0.066	0.064
	Pagimana	0.086	0.000	0.092	0.105	0.100
	Raja Ampat	0.066	0.092	0.000	0.106	0.062
	BiakNabire	0.066	0.105	0.106	0.000	0.092
	Ternate	0.064	0.100	0.062	0.092	0.000
Pooled	Wakatobi	0.000	0.071	0.067	0.051	0.075
	Pagimana	0.071	0.000	0.070	0.091	0.083
	Raja Ampat	0.067	0.070	0.000	0.085	0.058
	BiakNabire	0.051	0.091	0.085	0.000	0.083
	Ternate	0.075	0.083	0.058	0.083	0.000

In addition to the cluster analysis, the proximity population grouping could also be analyzed using discriminant analysis. The discriminant analysis presents plot distribution of morphometric characters from all sampling locations as well as its centroid. Figure 16 presents that the population of males Leopard coral grouper from Raja Ampat and Ternate, Pagimana and Biak-Nabire, and Wakatobi were separated each other, while in females the population grouping was not appear significantly. Moreover, if we only look at the location in the Fisheries Management Area (FMA) 715 (Pagimana, Ternate, Raja Ampat), it is known that the population of Leopard coral grouper create two distinct population, i.e. Pagimana population and combined population of Ternate and Raja Ampat.

According to the group prediction, it is known that some fishes show the similarity to others location. This phenomenon provides in the Table 17, where one fish of males Leopard coral grouper population from Biak-Nabire has the same characters to the fish population of Pagimana and Wakatobi. One fish from Pagimana has the similarity to the fishes from Biak-Nabire. One fishes of Ternate populations belongs to the population of Raja Ampat. Meanwhile, one fish of from Wakatobi has the similarity to each of Biak-Nabire, Pagimana and Ternate population. Group prediction based on the morphometric characters from the others locations present in the Table 18.

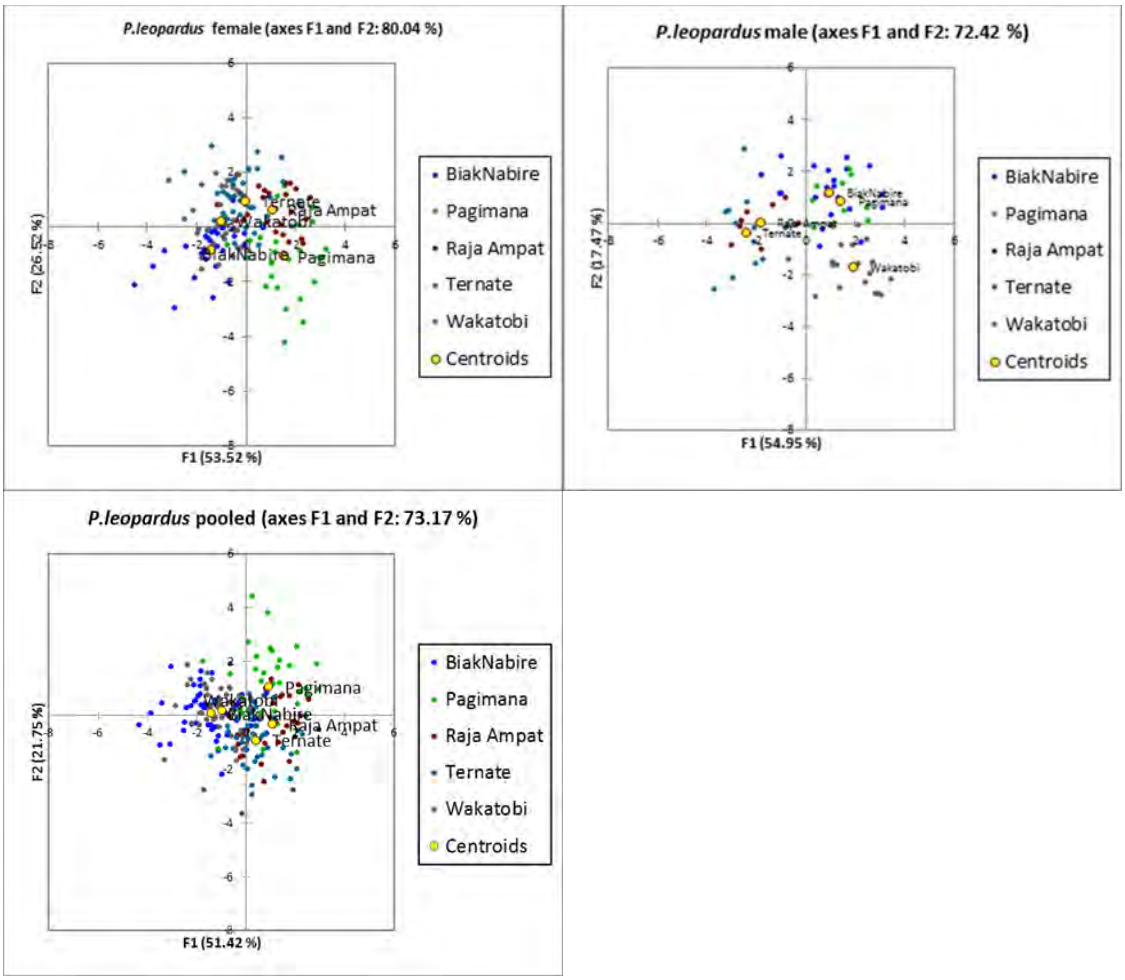

Figure 16. Morphometric characters distribution plot of *P. leopardus* in seven locations in eastern part of Indonesia waters

Table 18. Group prediction among sex of *P. leopardus* in sampling location

	Location	BiakNabire	Pagimana	Raja Ampat	Ternate	Wakatobi	Total	correct
Male	BiakNabire	12	1	0	0	1	14	81.82%
	Pagimana	1	13	0	0	0	14	90.00%
	Raja Ampat	0	0	14	0	0	14	100.00%
	Ternate	0	0	1	13	0	14	91.67%
	Wakatobi	1	1	0	1	11	14	78.95%
	Total	14	16	15	14	13	71	88.49%
Female	BiakNabire	21	1	0	3	5	30	68.97%
	Pagimana	0	23	4	2	1	30	75.86%
	Raja Ampat	1	5	17	6	1	30	56.00%
	Ternate	1	2	5	18	5	30	59.46%
	Wakatobi	3	1	0	6	21	30	68.75%
	Total	26	32	26	35	33	152	65.81%
pooled	BiakNabire	27	3	3	6	8	45	58.49%
	Pagimana	1	24	9	7	3	45	53.85%
	Raja Ampat	1	12	19	11	1	45	42.42%
	Ternate	3	6	8	22	6	45	48.00%
	Wakatobi	6	2	1	7	30	45	65.38%
	Total	38	47	40	53	49	227	53.63%

4.4. DISCUSSION

Morphometric is one kind of morphological characters mostly used to describe the variety of the exploited fish stocks (Saunders et al. 2009; Siddik et al. 2016). The results obtained from this present study indicated that there are significant phenotypic variations of Malabar blood snapper and Leopard coral grouper amongst sampling locations as well as between the sexes in the area of study. The Kruskal-Wallis test showed that all morphometric characters of those species were significantly different with each other of the sampling populations. Indeed, it is often fairly hard to describe the actual reason for morphometric variation between stocks. However, several studies reveal that variations occurred because of the different of environmental conditions, and geographic positions, and sometimes human error during the work on morphometric measurements and meristic counts (Cabral et al. 2003; Díaz de Astarloa et al. 2011; Chaklader et al. 2016).

The cluster analysis of Malabar blood snapper indicated that there are three distinct stocks, viz.: 1) Raja Ampat; 2) Kendari and Pagimana; and 3) Bau-bau, Ternate, and Tarakan. This clustering also confirmed by the discriminant analysis where the stocks from Tarakan, Ternate, and Bau-bau are not statistically different. Considering that phenotypical characteristics of individuals are affected not only by genotype but also environmental conditions, the results should be evaluated further based on genetic and environment information, since the location of Tarakan waters is separated from Ternate and Bau-Bau waters by Sulawesi Island. On the other hand, the population from Pagimana, and Raja Ampat were indicated as different groups of stock. The close morphological characteristics of Malabar blood snapper among Bau-bau, Ternate and Tarakan possibly caused by the human error when sampling in Bau-bau. It is strongly suspected that Malabar blood snapper which were landed in Bau-bau came from Ternate, Tarakan and the others location since the Malabar blood snapper samples bought from the large collectors.

Furthermore, from the point of view location in Fisheries Management Area (FMA) 715 which represented by Pagimana, Ternate, and Raja Ampat, it is known that the stocks of Malabar blood snapper from these three locations were separated each other. This phenomenon related to the behavior distribution of snapper, mostly lutjanid live in shallow to intermediate depth (<100 m) and the home-range of Malabar blood snapper on the depth of 12-100 m (Martinez-Andrade 2003).

The study on the Leopard coral grouper also showed three different stocks, that are: 1) Wakatobi and Biak-Nabire; 2) Pagimana; and 3) Raja Ampat and Ternate based on the cluster and discriminant analysis. However, Leopard coral grouper from representative location of FMA 715 showed that there are two distinct stocks, Pagimana in the western and Ternate-Raja Ampat in the eastern part of FMA 715. The closer morphological characteristic between Ternate and Raja Ampat could be explained by the oceanography phenomenon where the Halmahera Eddy transporting the water mass from northern part of Papua into the southern and the western part of Halmahera Islands (Kashino et al. 2013). This condition causes a mixture of Ternate and Raja Ampat Leopard coral grouper population which is driven by the larval dispersal of this species. Larval dispersal phenomenon is also thought to be occurred between the population of Wakatobi and Biak-Nabire.

4.5. CONCLUSION

The Malabar blood snapper in this study area was consisted of three stocks, i.e 1) Raja Ampat and Biak-Nabire; 2) Kendari and Pagimana; and 3) Bau-bau, Ternate, and Tarakan. The Leopard coral grouper was clustered into three different group, viz. 1) Wakatobi and Biak-Nabire; 2) Pagimana; and 3) Raja Ampat

and Ternate. According to the sampling location within FMA 715 ((Pagimana, Ternate, and Raja Ampat), it was revealed that population of Malabar blood snapper was separated each other. Meanwhile, Leopard coral grouper in those area was consisted of two distinct stocks, namely Pagimana and Ternate-Raja Ampat.

5. GENERAL DISCUSSION

Fish stock is one of the important biological information which is needed to understand since it is not only provide the characteristic and the origin of the stock but also the information that can be used in fisheries management. According to Stephenson (1999) stock identification is an important initial step in fisheries management. Begg et al. (1999) define the stock identification as an integral component of modern fisheries stock assessments, and in turn, for effective fisheries and endangered species management. To manage a fishery effectively, and implement stock rebuilding programs, it is important to know the identity of the stock structure of a species, and how fishing effort and mortality is distributed amongst the various components, as each stock must be managed separately to optimize their yield (Grimes et al., 1987). Disregard of stock structure and ineffective fisheries management can lead to dramatic changes in the biological attributes and productivity rates of a species, as well as the genetic diversity of a species (Smith et al., 1991).

There are many definitions and uses of the word "stock" (Smith et al., 1990), but there is no universally applicable definition of the term "stock" (Carvalho & Hauser, 1994). According to MacLean & Evans (1981), the stock concept contains two basic principles: that fish are subdivided into local populations, and that there are genetic differences between local populations which are adaptive. The simplest terms of stocks for management purposes are described both the availability of fish and the consequence of fishing itself. The stocks are locally accessible fish resources in which fishing pressure on one resource has no effect on the abundance of fish in another contiguous resource (Gauldie, 1988), or all of the fish that are within the range of the fishing vessels under the jurisdiction of a particular fisheries manager (Gauldie, 1991). Sparre & Venema (1998) define a stock as a subset of one species having the same growth and mortality parameters and inhabiting a particular geographic area. Meanwhile, Hindson et al. (2005) define stock as a group of individuals in a species occupying a well-defined spatial range, independent of any other stocks of the same species, that can be regarded as a single 'unit' for management or assessment purposes.

The result of this study using genetic analysis of mtDNA revealed that each of *L. malabaricus* and *P. leopardus* from the three sampling locations in FMA 715 came from the same population or have the same origins. However, it does not mean that they belong to the same stock, since the mtDNA is a genetic marker inherited from the maternal (female) line only (Antoniou & Magoulas, 2014).

The possibility that *L. malabaricus* or *P. leopardus in* FMA 715 consisting of more than one stock is indicated by the result of morphometric study of *L. malabaricus* or *P. leopardus in* FMA 715 reported here. The result of the morphometric analysis showed that the Malabar blood snapper in FMA 715 consisted of three stocks, i.e. 1) Raja Ampat; 2) Pagimana; and 3) Ternate. Meanwhile, the Leopard coral grouper in FMA 715 was clustered into two different groups, i.e. 1) Pagimana; and 2) Raja Ampat and Ternate.

Based on the definition that the stock is an intraspecific group of randomly mating individuals with temporal and spatial integrity (Ihssen et al., 1981; Smith et al., 1990; Donovan, 1991), genetic characteristics of fishes that are inherited from male and female parents are required to decide the unit of stock. The DNA source used for this analysis is DNA from the cell nucleus, or commonly called nucleus DNA. In the cell nucleus DNA there is information that is uniparental so that it can identify the diversity of DNA and stock units. One method that is often used was using microsatellite markers. Microsatellite markers are useful for population genetic studies because many are considered highly polymorphic. If a microsatellite locus is polymorphic, it means that there is more than one potential allele at a single locus (a specific marker site). Polymorphic loci can have more than 10, even more than 20 potential alleles in that given population. If populations are truly separate from each other, then these alleles are likely to be present in different frequencies in each population. These different allele frequencies increase the potential to observe genetic differences between populations if they exist. Abdul-Muneer (2014) stated that the very high levels of variability associated with microsatellites, the speed of processing, and the potential to isolate large number of loci provide a marker system capable of detecting differences among closely related populations. Furthermore, Abdul-Muneer (2014) revealed that the major advantages of microsatellite markers are codominant transmission (the heterozygotes can be distinguished from homozygotes), locus-specific in nature, highly polymorphic and hypervariable, high information content and providing considerable pattern, relative abundance with uniform genome coverage, higher mutation rate than standard and easy to sample preparation.

While completing scientific information, including further information from genetic research, management of the fishery targeting *Lutjanus malabaricus* and *Plectropomus leopardus* in FMA 715 should be carried out. As discussed previously, treating those fish species in three fishing areas as a single stock created risk of management failure if they actually belonged to different stocks, which have different growth, maturity, and mortality levels. The utilization of those stocks would be sub-optimal from implementing the same management strategy as practiced in the management of a single stock. Three different stocks may require three different management strategies to effectively achieve optimal utilization. Furthermore, those species distribute inside of the area under management responsibility of relevant provincial governments, and harvested by small scale fishery operated by local fishers living in the village close to each fishing area. To effectively controlling fishing intensity, it would be more effective if the fishery was managed by relevant provincial governments. The provincial government should develop a management strategy for each relevant fish stock.

One challenge to ensure sustainability and optimal harvest of the fish stocks was effective fisheries management. Management measures that can be implemented to control fishing mortality are output control, input control, and technical measures (FAO, 2003). Considering monitoring capacity of the local government of the provinces in FMA 715, it will not effectively implement output control. Control of reef fishery inputs can be carried out by reducing the level of fishing effort so that fishing pressure on reef fishery resources decreases.

Reducing the level of fishing effort, among others, can be done by regulating the number of fishing days and/or closing the season during the spawning season. Controlling the fishing effort through reducing the number of vessels would be inappropriate, because snapper and grouper fishing activities are the main source of income for most fishermen operating small-scale fisheries in FMA 715. Reducing the number of

boats will create un-employment, and in the short-term will reduce the welfare level of most of the fishing families. In addition, reducing the number of vessels will also have an impact on the socio-economic conditions of fishermen. Therefore, the reduction of fishing effort is carried out by reducing the time and location of fishing, as well as making the function of marine protected areas more effective (Campbell et al., 2014).

The pressure on snapper and grouper fishing in FMA 715 is not only a result of the operation of the local fishing fleet but also a result of illegal and destructive fishing practices by fishermen from outside the region. Therefore, reducing fishing effort should not only be undertaken by reducing the time and location of fishing, including making the MPA function more effective, but also by eradicating illegal and destructive fishing practices. With limited capacity of the local government to ensure compliance of fishers, the role of local fishers in surveillance-based community participation would be required. Fishing community members would be expected to observe during their presence at sea during fishing, and to report to surveillance officers at port when they found an indication of illegal fishing practices.

6. CONCLUSIONS

(1) Results of population genetic analysis using mtDNA markers indicate that Malabar blood snapper and Leopard coral grouper from Pagimana, Ternate and Raja Ampat locations are from the same origin.
(2) The Malabar blood snapper and Leopard coral grouper population from Ternate are more related to Raja Ampat than Pagimana population.
(3) Morphometric analysis results show that according to the sampling location within FMA 715 (Pagimana, Ternate, and Raja Ampat), it was revealed that the population of Malabar blood snapper was separated from each other. Meanwhile, the Leopard coral grouper in those areas was composed of two distinct stocks, namely Pagimana and Ternate-Raja Ampat.
(4) The Malabar blood snapper from Kendari - Baubau has a close kinship with fish from Tarakan, it is suspected that process of transshipment was occurred from Tarakan to Kendari – Baubau
(5) The result of this study using the mtDNA analysis cannot be used as a basis to conclude that *L. malabaricus* or *P. leopardus* in FMA 715 belong to the same stock. Further research needs to be undertaken to analyze genetic markers on the cell nucleus DNA.
(6) The management of the fishery targeting *Lutjanus malabaricus* and *Plectropomus leopardus* in FMA 715 should be carried out without waiting for complete scientific information. To effectively controlling fishing intensity, each provincial government should manage the fishery in its fisheries management area.
(7) To complement input control, reducing the time and location of fishing, as well as making the function of marine protected areas more effective, and elimination of illegal fishing practices should also be conducted.

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