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# GENETIC DIVERSITY OF MACKEREL SCADS, *Decapterus macarellus* (Cuvier, 1833) IN THE INDIAN OCEAN

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**ABSTRACT**

Mackerel scads (Decapterus macarellus) is a small widely distributed pelagic species in ocean. In 2013, monthly catch and abundance index of mackerel scads increased in western part of Sumatera waters. High exploitation of mackerel scads may lead to decrease stock due to the over exploitation. Stock information is very useful for calculating of the potential fish. Genetic analysis is one of the powerful tools to estimate fish stock quickly. Genetic diversity of mackerel scads in this study was analyzed using RFLP (Restriction Fragment Length Polymorphism) with AfaI, EcoR I, HapII, HinfI and TaqI restriction enzyme. The results showed that the lowest genetic diversity of mackerel scads was Labuan population. Kinship Labuan was also the furtherest stock compared to other populations. It can be concluded that the population of Labuan is derived from a different sub-species. The closest kinship was between Aceh and Sibolga stock.

**Keyword: Mackerel; Decapterus; genetic; DNA; Indian Ocean**

## INTRODUCTION

Mackerel scads or malalugis (*Decapterus macarellus*), is a high economic small pelagic species widespread in the deep sea of the Indian Ocean e.g. Western and Eastern part of Indonesia (Hariati, 2005). The exploitation of mackerel scads is now already overfishing and uncontrolled (Suwarso *et al*., 2000). Mostly purse seine is used in West Aceh, Sibolga and Kupang, lift net is also used to catch juvenile in Sibolga. In 2013, monthly catch and abundance index of mackerel scads in the west of Sumatera waters was 17,226 ton higher than 2012 which was 16,782 ton.

Over exploitation of mackerel scads in the Indian Ocean still exist while the data of potential production is not available. Stock unit of mackerel scads in the Indian Ocean is very important to understand their relationship and origin. A common method in fishery to clarify fish stocks is the analyses data of the following parameters: length, frequency distribution, length weight relationship, condition factor, age, growth, mortality and the rate of exploitation. Genetic analysis is one of the powerful tools to estimate fish stock quickly (Utter *et al.*, 1987), especially for the inter-regional management units of important marine species (Giles *et al.,* 2014). Santos *et al.* (2010) stated that the genetic diversity is enables to determine the status of the population.

Stock maintaining is very important for sustainable yield and conservation of genetic resources. Fisheries management based on the population genetic structure approach is needed. Mt-DNA genome of fish has a high degree of polymorphism containing information related to population unit, phylogeographic, migration patterns, hybridization and systematic and barcode (Ferris & Berg, 1987; Bermingham, 1990). Various fragment DNA analysis is applied now and small sequences is more preferred. Specific site of mt DNA is resulted from restriction enzymes work producing polymorphic profile for relationships analysis. The purpose of this study was to analyses the population diversity of mackerel scads (*Decapterus macarellus*) in the waters of the Indian Ocean using RFLP (Restriction Fragment Length Polymorphism) DNA method.

## MATERIALS AND METHODS

### Sampling

Sampling was conducted at four fish landing sites: 1) Kupang, South of Kupang; 2) Labuan, Sunda Strait; 3) Sibolga, West of Sibolga; and 4) Lampulo, West of Aceh (Figure 1). Twenty four mackerel scads were

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collected at each site, total 96 samples. Approximately 1 x 1 x 1 cm muscle from dorsal part was collected from each fish. The samples were preserved in appendorf tubes containing 96% ethanol and then were stored at room temperature for further DNA analysis at genetics laboratory of Research Institute for Marine Fisheries.


Figure 1. Sampling sites of mackerel scads (*D. macarellus*) in Indian Ocean.

**Sample Analysis**

The analysis was performed by using RFLP (Restriction Fragment Length Polymorphism) method (Fatchiyah *et al*., 2011). Extraction and purification process were implemented by the method of mini-column from PureLink™ Genomic DNA Kits, Invitrogen™. The amplification (PCR) process using the primer of HN20= 5'–GTGTTATGCTTTAGTTAAGC–3', and LN20 = 5'–ACCACTAGCACCCAAAGCTA–3'. Cycle on the PCR process set up as follows: 1 cycle of denaturation at 94 ° C for 2 min, 35 cycles of doubling consisting of 94 ° C for 1 min, 48 ° C for 1 minute and 72 ° C for 1 minute; and then the last cycle at 72 ° C for 5 minutes. Restriction enzymes are used consisting of 5 types, Afa I, EcoR I, Hap II, Hinf I and Taq I. Restrictions made to the sequences amplified from mitochondrial DNA (mtDNA) D-loop control region.

**Data Analysis**

The results of each restriction enzyme on individual samples (96 samples) obtained types haplotype. Types haplotype of each sample was analyzed by software TFPGA (Tools For Population Genetic Analyzes) (Miller, 1997) which includes the analysis of haplotype diversity, kinship between populations through pairwise distance test (FST), a distance of genetics and kinship between populations are presented in the form of a dendrogram. Haplotype diversity analysis conducted by Nei & Tajima (1981) by the equation:

$$h = \frac{n}{n-1}\left(1 - \sum X_i^2\right) \quad \text{.....(1)}$$

where;
h = haplotype diversity
n = number of samples
$X_i$ = haplotype frequency samples

Genetic distance is a measure of genetic differences between populations were calculated based on the frequency of haplotypes per population. The calculation of genetic distance by Nei & Tajima (1981) through the TFPGA by the equation:

$$D = - \ln \{J_{ab}/\{(J_a \times J_b)^{0,5}\}] \quad \text{.....(2)}$$

where;
D = Genetics distance
$J_{ab}$ = Loci haplotype frequencies in the same population
$J_a$&$J_b$ = Haplotype frequencies in populations A and B

Dendrogram kinship between populations based on haplotype diversity, kinship between species in the population, kinship between populations through pairwise distance test (FST) and cluster analysis of the genetic distance value according to the average distance method UPGMA (Unweight Pair Group Methods Arithmatec) (Bermingham, 1990) by using software TFPGA (Miller, 1997).

## RESULTS AND DISCUSSION

### Results

Electrophoresis of five restriction enzymes produced 13 genotypes of DNA fragments (Table 1). The result of Taq I enzymes is in Figure 2 showing three restriction sites.

Genotype of mackerel scads is presented in Table 2 and it is known that for all enzymes used to produce DNA fragments type A and B. C type fragments are only produced by the EcoR V and Taq I enzymes.

Based on the obtained restriction sites, nine genotypes of alleles/haplotypes were recognized as AAAAA, AAABA, ABAAA, ABABA, ACAAA, ACAAB, ADAAA, BBAAC and BBBAC. Haplotype diversity ranged from 0.0310 to 0.2522 and mean was 0.1667 (Table 3).

Table 1. Genotypes of DNA resulted from five restriction enzymes Afa I, EcoR V, Hap II, Hinf I dan Taq I.

| Length of Fragment | Enzyme | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Afa I | | EcoR V | | | | Hap II | | Hinf I | | Taq I | | |
| (bp) | A | B | A | B | C | D | A | B | A | B | A | B | C |
| 800 | | | | | | | | | | | | | |
| 750 | | | | | | | | | | | | | |
| 700 | | | | | | | | | — | | | | |
| 650 | | | | | | | | | | | | | |
| 600 | | | | — | | | | | | — | | | |
| 550 | | | | | | | | | | | | | |
| 500 | | — | | | | | | | | | | | |
| 450 | | | | | | | — | — | | | | | |
| 400 | | | | | | — | | | | | — | | |
| 350 | — | | — | | | | | | | | | | — |
| 300 | | | | | — | | | | | | | | |
| 250 | | | | | | | | | | | | | |
| 200 | — | | | | | | — | | | | | — | |
| 150 | | | | — | | — | | | | — | — | — | |
| 100 | — | — | | | — | | | — | | | — | — | |
| 50 | | — | — | | — | — | — | — | — | | | | — |
| Number or fragment | 4 | 4 | 3 | 2 | 4 | 4 | 4 | 5 | 2 | 2 | 4 | 5 | 3 |
| N | 72 | 24 | 34 | 54 | 6 | 2 | 94 | 2 | 74 | 22 | 70 | 2 | 24 |

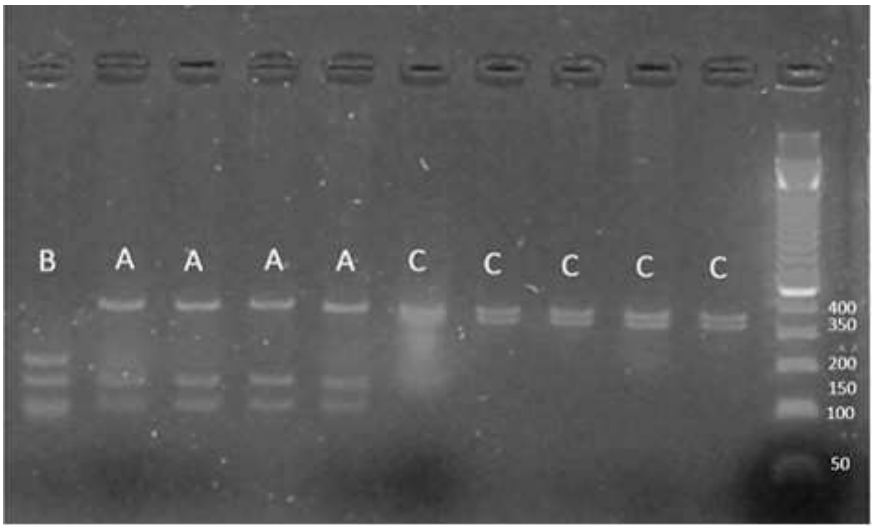


Figure 2. Results of restriction of mackerel scads DNA from Labuan with Taq 1 enzyme (three types of restriction A, B and C).

Table 2. Genotype distribution (type of restriction) in four Mackerel scads populations

| Enzyme | Type of restriction | Kupang | Sibolga | Labuan | Aceh |
|---|---|---|---|---|---|
| **Afa I** | A | 24 | 24 | | 12 |
| | B | | | 24 | |
| **EcoR V** | A | 16 | 10 | | 8 |
| | B | 6 | 12 | 24 | 12 |
| | C | 2 | 2 | | 2 |
| | D | | | | 2 |
| **Hap II** | A | 24 | 24 | 22 | 24 |
| | B | | | 2 | |
| **Hinf I** | A | 20 | 14 | 24 | 16 |
| | B | 4 | 10 | | 8 |
| **Taq I** | A | 24 | 22 | | 24 |
| | B | | 2 | | |
| | C | | | 24 | |

Table 3. Haplotype of the mtDNA D-loop of mackerel scad (*Decapterus macarellus*) in the Indian Ocean

| No. | Composite Haplotype | Haplotype Freq. | | | |
|---|---|---|---|---|---|
| | | Kupang | Sibolga | Labuan | Aceh |
| **1** | AAAAA | 0.58 | 0.417 | | 0.333 |
| **2** | AAABA | 0.083 | | | |
| **3** | ABAAA | 0.167 | 0.083 | | 0.167 |
| **4** | ABABA | 0.083 | 0.417 | | 0.333 |
| **5** | ACAAA | 0.083 | | | 0.083 |
| **6** | ACAAB | | 0.083 | | |
| **7** | ADAAA | | | | 0.083 |
| **8** | BBAAC | | | 0.917 | |
| **9** | BBBAC | | | 0.083 | |
| **N-alelle** | | 5 | 4 | 2 | 5 |
| **Haplotype Diversity** | | 0.1594 | 0.2522 | 0.0319 | 0.2232 |

Pair wise distance method ($F_{ST}$), showed that there is a significant different between Labuan population with others from Kupang, Sibolga and Aceh (Table 4). Genetic distance showed that Labuan population has furthest relationships among populations observed (Table 5 and Figure 3).

Table 4. Inter population analysis of *Decapterus macarellus* pair wise distance method

| | Kupang | Sibolga | Labuan | Aceh |
|---|---|---|---|---|
| Kupang | ***** | | | |
| Sibolga | $0.5548^{ns}$ | ***** | | |
| Labuan | $0.0000^{s}$ | $0.0000^{s}$ | ***** | |
| Aceh | $0.6456^{ns}$ | $0.9854^{ns}$ | $0.0000^{s}$ | ***** |

*Remarks*: ns: not significantly different (P>0,05); n: significantly different (P<0,05)

Table 5. 'Nei' genetic distance of *Decapterus macarellus* in the Indian Ocean.

| | Kupang | Sibolga | Labuan | Aceh |
|---|---|---|---|---|
| Kupang | ***** | | | |
| Sibolga | 0.0319 | ***** | | |
| Labuan | 0.8179 | 0.7625 | ***** | |
| Aceh | 0.0286 | 0.0052 | 0.7396 | ***** |

Genetic relationship showed that the four populations can be separated into two groups, the first group consists of a population of Sibolga, Aceh and Kupang and the second one was only Labuan population Labuan.

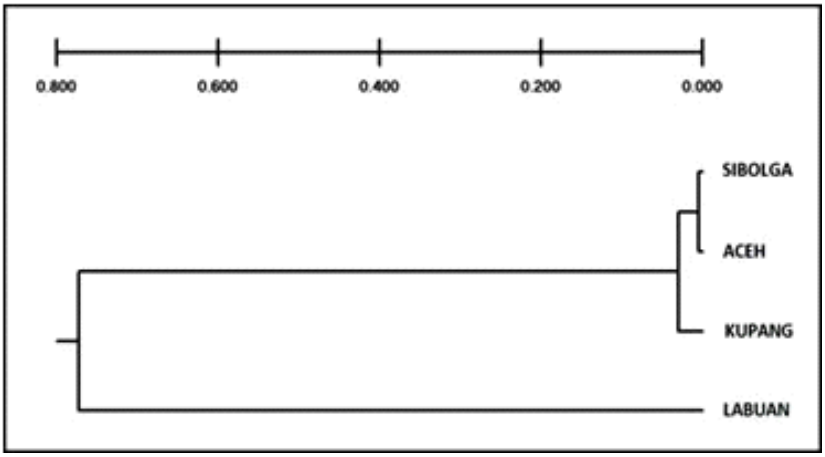


Figure 3. Dendrogram phylogeny of 4 populations of *D. macarellus* in Indian Ocean.

**Discussion**

Kinship *Decaptarus macarellus* from four populations showed that the population of Aceh, Sibolga and Kupang included in one group of the population, while the population of Labuan included in another group. Genetically the population of Labuan differs from the other three populations. The distance of Aceh and Sibolga is geographically close, yet far enough away from the location of Kupang, but still allow the gene flow from Kupang to Aceh and Sibolga (west coast of Sumatera). Mackerel scads is small pelagic fish that is oceanic, and migrate widely. Allegedly the location of Aceh, Sibolga and Kupang is the migration path of scads mackerel in the waters of the Indian Ocean. Allegedly Mackerel scads in Labuan originated from the Indian Ocean that settled in the waters of the Sunda Strait and genotypically adaptation occurred. The waters of the Sunda Strait is a dynamic strait. The northern part of the strait which is directly related to the Java Sea has the dominant influence of water masses from the Java Sea and South China Sea, while in the southern part of the waters is strongly influenced by the conditions of the Indian Ocean waters (Wyrtki, 1962). According to Birowo (1983) Sunda Strait lies between the island of Sumatra and Java Island where the mass of Java Sea water mixed with the mass of water from the Indian Ocean. This causes the fish to adapt, causing genotyping genes that were originally mutations in only one individual of the individual population constituents to undergo change (Sofro, 1994). The migration process is influenced by two factors: internal and external. Internal factors include the form of metabolic equilibrium such as migration for food and external factors including the current, temperature and salinity. Susanto *et al*. (2001) stated that in the month of June to October upwelling occurs along the waters south of Java to western of Sumatra. This upwelling process is a response to the monsoon cycle. In June, the southeast monsoon winds cause upwelling and the erosion of the thermocline in the waters of East Java. The upwelling spread westward to the tip of Sumatra and moved closer to the equator during the months of June to October. Wyrtki (1961) suggested that in July to October, East Monsoon Winds push the South Equatorial Currents (SEC) to the north. SEC were pushed to the north in the period suspected of spreading mass of cold water and high salinity coming from upwelling spreading further north.

According to Pfenninger *et al.* (2011) factors potentially shaping genetic diversity are geographical marginality, environmental marginality, habitat size, potential biotic interactions, range expansions and dispersal barriers. Liu *et al*. (2013) suggest that ecological factors can have strong impacts on both population size per se and intrapopulation genetic

variation even at a small scale. On a more general level, our data indicate that a patchy environment and low dispersal rate can result in fine-scale patterns of genetic diversity. The number of types of composite haplotypes effect on genetic diversity in a population, the more the number of types of composite haplotype diversity will be higher, and vice versa. The value of genetic diversity can be seen from the value of haplotype diversity. Haplotype diversity value obtained from this study is 0.0319 to 0.2522, these values lower than studies conducted in the waters around Sulawesi for the same species, which ranges from 0.3698 (Zamroni *et al*., 2014), and lower than other marine fish species, such as yellow fin tuna in Maluku is 0.984 to 1.00 (Akbar *et al*., 2014) and grouper at 0.7749 to 0.7940 (Sembiring *et al*., 2013). According Avise *et al*. (1989) in Tabata *et al*. (1997) mention that the whole mtDNA haplotype diversity for some fish are in the range of 0.473 to 0.998. Low genetic diversity (haplotype diversity) in this study because the sampling sites are interconnected (Southern Indian Ocean, Sunda Strait and the Indian Ocean west of Sumatra). Fishing pressure on mackerel scads fish can also cause a reduction in the genetic diversity of a species. According to Wilson & Clarke (1996), the increasing exploitation and pressure on the environment can lead to a decline in stock abundance and average size of the fish; adverse genetic selection against potential fecundity; reducing the average size of the spawn; changing the sex ratio and balance interspecific; and the loss of genetic diversity. High genetic diversity in the population will have a better chance of survival because each gene has a different response to environmental conditions. Hartl & Jones (1998) stated that the high genetic diversity in the population of fish may protect from environmental interference.

The lowest number of composite haplotype is the population of Labuan which consists of two types, while the highest number of composite haplotype is belong to Kupang and Aceh populations, which consists of five types. Low composite haplotypes / genetic diversity in Labuan is apparently due to the exploitation of mackerel scads fish intensified, and the condition of the Sunda Strait which is less wide (narrow). The results of Octoriani, (2015) is study show that the actual exploitation of multispecies in the Sunda Strait caught in purse seine has already occurred over exploitation. The results are supported by a low survival rate, which is suspected because of the high pressure of catching. The length of first captured (Lc) is smaller than the length of the first mature gonad (Lm). The optimal effort (EMEY) result of bioeconomic analysis of competition is smaller than EMEY without considering the inter-species relationship. The results of the bioeconomic analysis show that species of Goldstripe sardinella, mackerel, neritic tuna, and scads are indicated to have undergone biological overfishing and economic overfishing. Exploitation may lead to an increased rate of genetic drift, in addition to the small population tends to occur inbreeding, which can adversely affect the survival of the population. The indication is the decline in the genetic diversity of the population, namely the decline in haplotype diversity and nucleotide diversity (Zein, 2007). A decrease in the genetic variability could endanger the survival of the population because it can reduce the ability of the individual in the face of natural selection pressures, mainly due to changes in the environment (Hedrick, 2000). Allendorf *et al*. (2008) said that catching the fish populations potentially against the three genetic changes: changing population structure, genetic variation selective genetic. Dammannagoda *et al*. (2011) said that to maintain the productivity of the fish population, it is imperative to incorporate the genetic consideration in fisheries management. The management plan should be developed by applying the basic principles of genetics combined with molecular genetic monitoring to minimize harmful genetic changes. Understanding the genetic changes and evolutionary response of the exploited population is also important to design management strategies aimed at sustainable use of biological resources (Walsh *et al*., 2006). This study shows that there are two main populations structures, so that, in the management of mackerel scad fish, it is recommended to use a different method to approach each population group. Labuan populations that have low genetic diversity and narrow waters emphasis on conservation, for example, by regulation the fishing season and the closure of fishing areas. The population of Aceh, Sibolga and Kupang who have extensive water areas and higher genetic diversity of a population of Labuan. For that is expected in the waters of these three populations genetic diversity can be maintained by not exploiting small-sized fish. One way is to use fishing gear that has a high selectivity, for example, restrictions on mesh size and the size of the mouth of the trap.

## CONCLUSION

The genetic diversity of mackerel stock in Indian Ocean is low with the index in the range 0.0310 to 0.2522. The Indian Ocean consisted of two stock mackerel scads, the first stock is derived from three sites/location Aceh, Kupang and Sibolga, and the other stock from Labuan. The Indian Ocean is the migration path of mackerel scad.

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