

## Determination on Yellow Fin Tuna Stock (*Thunnus albacares*) In South Java Sea Based on Genetic Variation by Restriction Fragment Length Polymorphism (RFLP) Method

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### ABSTRACT

Indonesia is known as a country with the highest potential production of tuna, however, Tuna fishery have several challenges, as can be identify by the declining in productivity and an average length fish had been caught, and also tuna fishing grounds are getting farther. This apparently appears as a result of inaccurate determination of fish stock especially in yellow fin tuna (YFT). This study aims to define population stocks of YFT in South Java Sea based on genetic information. Samples were taken from three fish landing ports (Muara Baru, Cilacap and Benoa). Restriction Fragment Length Polymorphism (RFLP) technique were applied for *Cytochrome Oxidase I* genes, using restriction enzymes; *HaeIII*, *RsaI*, *BamHI*, *XhoI*, and *AluI*. Genetic variation in populations were evaluated with POPGENE 32 software. Result indicate that finer scale of stocks present YFT and should be managed differently for effective management purpose and to maintain sustainability of this species.

**Keyword:** Cyrochrome oxidase, genes, popgene, RFLP, Tuna.

### INTRODUCTION

Yellow Fin Tuna (*Thunnus albacares*) is oneof pelagic fish that own high demand by the export market widely. As highly commercial fish, there always increasing of Yellow Fin Tuna catching around the world including in Indonesia;s waters. Therefore, it

is necessary to manage sustainability of Yellow Fin Tuna resources. However, Sibagariang et al. (2011), in they study stated that the status of Yellow Fin Tuna fisheries in the waters of Cilacap, Central Java, Indonesia was in overfished condition (over fishing) with a utilization rate reached 85.12%. Basically utilization limits allowed byagreed Code of Conduct for Responsible Fisheries (CCRF) is approximately 80%.

Fisheries management may fail due to neglecting the importance of determination the status of the stock (management unit) of a species of fish (Hart and Reynolds, 2004). Inaccuracy in managing sustainable fisheries may cause the depletion of fish resources in their nature habitat. This occurrence has been observed recently that some fishery management in the world have fail to maintain the presence of fish resources in the wild (Beaumont *et al.*, 2010). As a basis of fisheries management, determining of fish stock (management unit) is necessary to ascertain the future sustainability of fisheries (Welch *et al.*, 2015)

For effective fisheries management, biological informations, such as population size, habitat and migration paths, age structure, reproduction patterns, natural mortality rate, and also the level of fishing effort should be considered (Beaumont *et al.*, 2010). Generally, morphological approach or physical characteristics have

been performed to determine stock of some fish populations. Generally, morphological approach or physical characteristics have been performed to determine stock of some fish populations. However, inability to assess gene flow of two or more individuals with morphological similarity is a weakness of this approach (De Queiroz and Gauthier, 1992). Thus, information of genetic structure will have significant consequences in determining management unit Yellow Fin Tuna (*Thunnus albacares*) in South of Java Sea.

One method can be used to determine genetic structure of fish populations is Restriction Fragment Length Polymorphism (RFLP). Asmanik (2003) have been revealed the genetic variation and population structure of *Metapenaeus monoceros* shrimps from three populations (Jember, Tuban and Makassar) this method. Previous genetic study on Big Eye Tuna fish has been done by Nugraha and Barata (2011). They suggested that there was an admixture of two stocks of Big Eye Tuna fish in the Indian Ocean, that may indicate the potential admixture stock is present for yellow fin tuna in the same geographical area.

This study aims to determine the population structure of yellow fin tuna (*Thunnus albacares*) of three populations (Jakarta, Cilacap and Bali) in South Java Sea and to reveal whether the population being observed can be managed as a single unit management based on their genetic variation. This genetic information is important for management purposes and for conservation of tuna fisheries resources (Beaumont et al., 2010).

## METHODS

### 1. Sampling Method.

Samples of yellow fin tuna fish were taken from three locations along the South

Java Sea. Fish were collected from fish landing sites namely Benoa Port (Bali), Ocean Fishing Port Cilacap (Central Java), and the Nizam Zachman Port (Jakarta). These location were chosen because the major production of tuna fishery in Indonesia were produced from these area (Nugraha and Hufiadi, 2013). As migration and spawning ground of tunas fish species, especially Yellow Fin Tuna, the Indian Ocean become has significant contribution to industrial-scale of tuna fisheries in Indonesia. Samples were collected from fish market in each ports, twenties individual per sites. Tissue samples (caudal fin or muscle) were taken using appropriate protocols. Tissue samples stored alcohol 96% in labeled vials and were kept in refrigerator temperature at -80°C for further genetic analysis. Sampling locations of Yellow Fin Tuna is presented in Figure 1.

### 2. DNA Extraction

Mitochondrial DNA extraction were performed using the chelex method 10% (Walsh *et.al.*, 1991; Akbar *et.al.*, 2014). Small amount (one hand pull tweezers) of tissue (muscle) samples were put in 1.5 ml *ependorf* tube contain 500 µl of 10% chelex 100. 5 µl proteinase kinase subsequently added to this tube and the mixture then vortex and incubated for 3-4 hours at 55°C in water bath until the muscle dissolved in the mixture (within that time, several vortexes were done to speed up the process). The mixture then incubated for 8 minutes in water bath at 89°C and cooled for 10 minutes at 20°C in refrigerator. 55 µl of Trish EDTA (TE) was added to the mixture and next steps were vortex and centrifuged for 5 minutes at room temperature at 13000 rpm. Supernatant was then transferred to a new 1.5 ml labelled *ependorf* tube properly and stored at -20°C in refrigerator.



Figure 1. Sampling Sites Location.

**3. Polymorphism Chain Reaction (PCR).**

PCR primer used for DNA amplification is universal primer Fish-BCH (5'-TCAACYAATCAYAAAGATATYGGCAC-3') and Fish-BCL (5'-ACTTCYGGGTGRCCRAARAATCA3') to amplify 700 bp of COI (Cytochore Oxidase I) gene of mitochondrial DNA (Baldwin *et.al.*, 2008). The COI were selected because previous genetic study in tuna was successfully amplified this gene (Chiang *et.al.*, 2008). PCR Amplification was performed in a total of 12 µl PCR volume contained of be 5 µl Dream Taq *master mixed*, 1 µl of forward primer (FBCH), 1 µl of reverse primer (FBCL), 1 µl of ddH2O and 4 µl of DNA template. PCR condition were initial denaturation at 95°C for 5 minutes, proceeded by 30 cycles comprising 1 minutes denaturation at 95°C, 1 minutes annealing at 55oC and 1 minutes elongation at 72°C. Success of PCR amplification was confirmed in a 150% agarose gel with 1x TBE (Trish buffer EDTA) and run for 120 minutes at 50 Volts and subsequently visualized under UV light.

**4. Restriction Fragment Length Polymorphism (RFLP).**

Single Band obtained from mtDNA PCR were cut with four types of *restriction enzyme* which are *HaeIII*, *RsaI*, *BamHI*, *XhoI*, and *AluI* (Lin *et.al.*, 2005; Kunal *et.al.*, 2015). RFLP was performed in a total mixture volume of 32 µl contained of 10 µl of products PCR, 18 µl of ddH2O, 2 µl of 10X buffer and 2 µl of enzymes restriction. The mixture was incubated in

incubator for 150 minutes at 37oC. Restriction result was confirmed in a 1.5% agarose gel in 1x TBE (Trish buffer EDTA) and run for 120 minutes at 50 Volts, with control was uncut of PCR products and subsequently visualized under UV light.

**5. Data Analysis.**

The target amplification product (COI gene) that has been cut with restriction enzymes then be coded or scored based on the presence or absence of restriction sites. Chow (1998) suggests that a single gene locus of amplification can be used to infer the number of alleles at each restriction enzyme profiles. Fragment that has no restriction site (uncut) can be confirmed as an A allele, while having one site can be confirmed as B alleles and so on (Ciftci and Okumus, 2002). Scored data used to calculate the population genetic structure of yellow fin tuna fish was performed by software popgene32 (Yeh *et.al.*, 1999).

**RESULT AND DISCUSSION**

**1. mtDNA Amplification**

mtDNA COI amplification applied Fish BCL and Fish BCH primer at 50°C annealing temperature generated single band (780 bp) for all samples as shown in Figure 2. The results of PCR showed that some of smear bands were appeared on sample 1, 3, and 15. This might be influenced by limited concentration of DNA templates (Tumanduk, 2014).

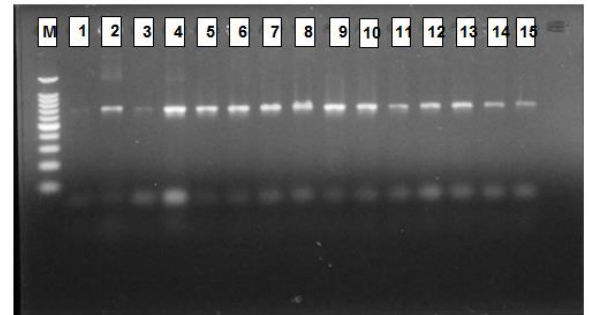
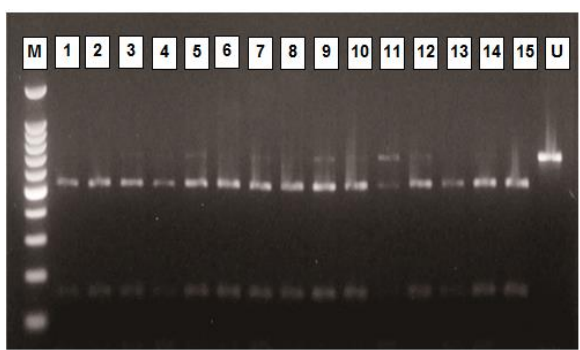


Figure 2. Gene COI amplification result (M: marker DNA ladder 100 bp; 1-5: samples from Jakarta; 6-10: samples from Cilacap; 11 -15: samples from Bali).

The RFLP results performed that restriction enzyme *HaeIII*, *AluI*, and *RsaI* successfully cut COI gene of mtDNA, while *BamHI* and *XhoI* could not recognize the restriction sites of COI gene. The results also showed that the polymorphic pattern was only identified by *RsaI* enzyme, while two other enzymes (*HaeIII* and *AluI*) had a monomorphic pattern. Below are shown restriction pattern of *RsaI* enzyme (Figure 3).



**Figure 3.** Restriction results of COI gene by *RsaI* enzyme (M: marker DNA ladder 100 bp; 1-5: samples from Jakarta; 6-10: samples from Cilacap; 11-15: samples from Bali).

This figure illustrated that *RsaI* enzyme had two to three polymorphic patterns on 705 bp, 676 bp, and 165 bp. The restriction patterns on sample 1, 2, 4, 6, 8, 13, and 15 were characterized as allele B while sample 3, 5, 7, 9, 10, 12, and 14 were confirmed as allele C and only one sample (sample 11) was identified as allele D (Çiftci and Okumuş, 2002). The further analysis applied POPGEN software version 1.32 to assess several population parameters.

**2. Population structure of *Thunnus albacares*.**

Several genetic parameters were taken into account to assess the population structure of *Thunnus albacares* in Southern Java Sea. They were allele frequency, heterozygosity, and F-statistic ( $F_{st}$ ,  $F_{is}$  dan  $F_{it}$ ) and genetic distance. Allele frequency is a proportion of each allele appeared in a population, which is shown in Table 1.

**Table 1.** Allele frequency of *Thunnus albacares* in five locus.

Allele	Locus				
	HaeIII	RsaI	BamHI	XhoI	AluI
A			1.00	1.00	1.00
B	1.00	0.467			
C		0.467			
D		0.07			

According to Çiftci dan Okumuş, (2002) a polymorphic locus is a locus with allele frequency less than 0.99. Therefore, *RsaI* enzyme was appropriate as a genetic marker than other enzymes (*HaeIII*, *BamHI*, *XhoI*, and *AluI*) since it can detect polymorphism in *Thunnus albacares*. The average heterozygosity of *Thunnus albacares* from all loci was 0.1067 which indicated that all population observed (Jakarta, Cilacap and Bali) had a closed genetic distance. The low value of heterozygosity might be caused by inbreeding in population of *Thunnus albacares* in South Java Sea (Mulliyadi and Arifin, 2010).

Below is F-statistic of *Thunnus albacares* from all loci.

**Table 2.** F-statistic of *Thunnus albacares*

Locus	$F_{is}$	$F_{it}$	$F_{st}$
HaeIII	****	****	0.00
RsaI	1.00	1.00	0.0476
BamHI	****	****	0.00
XhoI	****	****	0.00
AluI	****	****	0.00
Total	1.00	1.00	0.0476

**Note:**  $F_{is}$  : Fixation index within population.  
 $F_{it}$  : Total of fixation index.  
 $F_{st}$  : F statistic among population.

The value of  $F_{st}$  is an index of genetic difference that indicates the variation of allele frequency among populations (Beaumont *et.al.*, 2010). The table showed

that the  $F_{st}$  of *Thunnus albacares* in three observed populations was 0.0476. This indicated high levels of gene flow are present in YFT that minimize the differentiation between population and the populations can be assumed as single unit management (Hellberg, 2009).

*Fixation indexes* ( $F_{is}$  and  $F_{it}$ ) are determined by the total observed heterozygosity ( $H_{obs}$ ) and expected heterozygosity ( $H_{exp}$ ) following Hardy Weinberg equilibrium. In this study, the  $F_{is}$  was about 1.00 which indicated that there was a limited heterozygosity in the observed populations Holsinger dan Weir (2009). The  $F_{it}$  indicated that allele distribution is not equal for all populationn. As a consequence, the low genetic variation of *Thunnus albacares* in South Java Sea might diminish adaptation ability of this species to environmental changes (Çiftci dan Okumuş, 2002).

Regarding the population structure estimation, the genetic distance value of *Thunnus albacares* landed in Jakarta and those in Cilacap was about 0.0075. This value is higher than the genetic distance value of *Thunnus albacares* landed Jakarta and Bali which was only 0.0073. This indicated that *Thunnus albacares* landed in Jakarta has a closer relationship to those landed in Bali than those landed in Cilacap.

Generally, the genetic distance value of *Thunnus albacares* landed in the observed locations is lower than those in western Pacific Ocean and western Indian Ocean which was 0.423 Wu et.al. (2010). This implied that the observed populations might be originally from the same genetic pool.

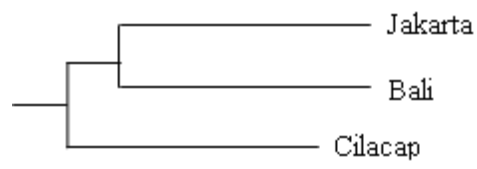
The genetic distance of *Thunnus albacares* in South Java Sea (landed in Jakarta, Cilacap, and Bali) are performed in Table 3.

**Table 3.** The genetic distance of *Thunnus albacores* in South Java Sea.

Population	Jakarta	Cilacap	Bali
Jakarta	-		
Cilacap	0.0075	-	
Bali	0.0073	0.0073	-

From table 3 population structures were clustered by population tree applied Unweighted Pair Group Method with Arithmetic Mean (UPGMA). Even though the genetic difference of *Thunnus albacares* landed in Jakarta – Cilacap (0.0075) and those landed in Jakarta – Bali (0.0073) is relatively low, it might be possible to classify those as two different management unit (Figure 4). The first management unit is *Thunnus albacares* landed in Jakarta and Bali, while the second one is those landed in Cilacap.

However, genetic analysis using nuclear DNA might produce different results. This analysis will perform genetic characteristic inherited from both male and female parents, while the mtDNA analysis will only expose maternal inheritance. Previous research by Palumbi dan Baker (1994), showed that both analysis gave different results of population structure of *Megaptera novaengliae* in North Atlantic, Antartic Ocean, Hawaii, Alaska, California, and Mexico.



**Figure 4.** Genetic Distance of *Thunnus albacares*.

**3. The environmental variability towards genetic variation of *Thunnus albacares*.**

According to Çiftci and Okumuş (2002), a population can experience with gene flows as a result of less geographical distance. Geographically, the fishing ground of

*Thunnus albacores* landed in Jakarta and Cilacap is relatively closer (519 km) compared to those landed in Bali (1080 km). This indicated that *Thunnus albacores* landed in Jakarta and Bali have a close genetic distance.

Sea surface temperature highly influence tuna distribution since temperature will directly affect water mass along with nutrient transportation (Sukresno et.al., 2015). According to Holland (1990), as a pelagic species, *Thunnus albacores* tend to swim in sea surface (at depth 50-90 m). Therefore, the difference of sea surface temperature can produce several genetic structures in the observed populations.

Regarding environmental variability such as distribution of sea surface temperature and klorofil-a (Figure 5), distribution of *Thunnus albacores* in Jakarta and Bali has some similarities. On the other hand, the environmental variabilities of *Thunnus albacores* fishing ground in Cilacap (around Central Java Province) changed more rapidly than those in Jakarta and Bali.

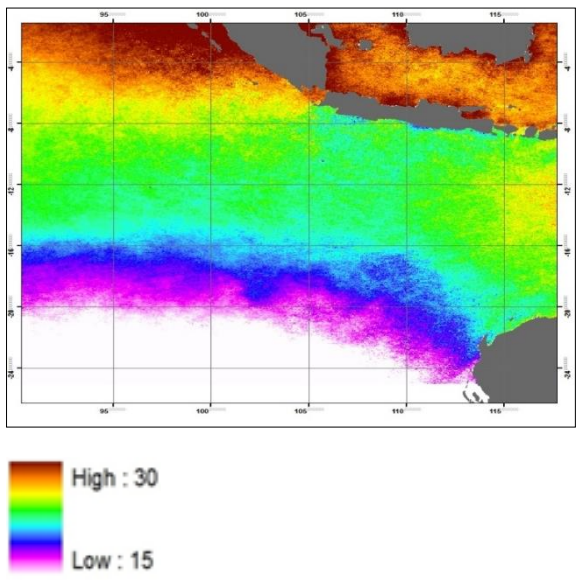


Figure 5a. Distribution of sea surface temperature in 2012.

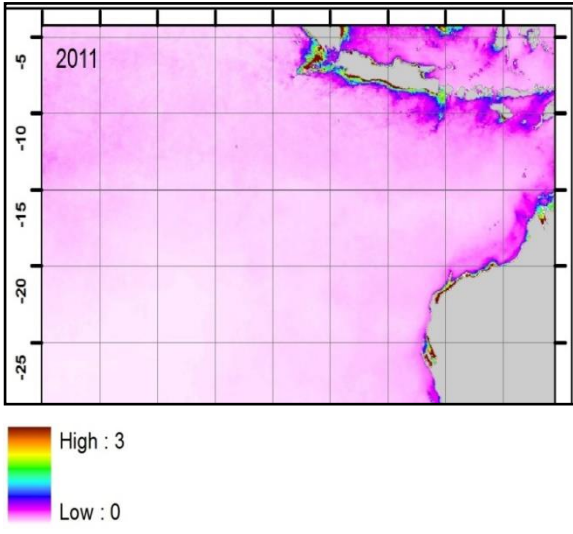


Figure 5b. Distribution of Chlorophyll-a in Indian Ocean 2011 (Ghafiky, 2016).

Genetic similarity of *Thunnus albacores* landed in Jakarta and Bali might possibly happen since their fishing grounds have similar environmental condition (Ghafiky, 2016). Regarding sea surface temperature condition, it has fluctuated more rapidly in Cilacap than those in Jakarta and Bali. In addition, distribution of klorofil-a in the observed areas were slightly different where the distribution of this nutrient in Jakarta and Bali than those in Cilacap. This might be caused by the existence of estuaries around fishing ground of *Thunnus albacores* in Jakarta and Bali which transport nutrients. Environmental changes results in local adaptation of population that genetically generate some differences compared to other populations.

As stated by Çiftci and Okumuş (2002), genetic variation is probable to happen in a species or a population for adaptation purpose towards its environmental changes. In this study, *Thunnus albacores* is a migratory species that their gene will response to any environmental changes. According to Nugraha dan Barata (2011), genetic variation in a population performs its heterogeneity based on space, even in

close geographical locations. Therefore, it might be possible if *Thunnus albacares* landed in Cilacap which is geographically close to those in Jakarta determined as two different management units.

The results of genetic variation successfully performed population structure of *Thunnus albacares* landed in the observed populations (Jakarta, Cilacap, and Bali). This will be applied to manage the tunas fisheries in those locations. As stated by Carvalho and Hauser (1994) and Beaumont et al. (2010), genetic information of a management unit will be beneficial for making fishery policies.

However, for fisheries management purposes, *Thunnus albacares* landed in Jakarta and Bali which classified as one management unit, it would be better if the stock managements are performed separately. It is because geographically and administratively, those populations are impracticable to be combined. Therefore, the fisheries management of *Thunnus albacares* landed in the observed areas determined as three different management units and their managements will be adapted to each area. If those *Thunnus albacares* populations suffer of overfishing in either Jakarta or Bali, those will respectively do restocking.

#### CONCLUSION AND SUGGESTION

1. The average of heterozygosity value of *Thunnus albacares* landed in Jakarta, Cilacap, and Bali is 0.1067 (inbreeding). The genetic distance value of *Thunnus albacares* landed in Jakarta-Bali (0.0073) is lower than those in either Jakarta-Cilacap or Bali-Cilacap (0.0075).
2. Based on genetic distances, it was determined that *Thunnus albacares* landed in Jakarta and Bali defined as a management unit, while those in Cilacap marked as a different management unit.

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