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POPULATION STRUCTURE AND REPRODUCTION OF BIG EYE TUNA (*Thunnus obesus*) IN INDIAN OCEAN AT WESTERN PART OF SUMATRA AND SOUTHERN PART OF JAVA AND NUSA TENGGARA

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ABSTRACT

This research was conducted to identify population structure and reproduction aspect of big eye tuna (*Thunnus obesus*) in west off Sumatra and south off Java and Nusa Tenggara of Indian Ocean. The sample of fish was collected from catch landed by fishermen. The fish was caught from the Indian Ocean during 2010. Analysis of population structure was done by using of histology technique of fish genetic and reproduction. Result showed that population structure of the big eye tuna in Indian Ocean consisted of two different sub populations namely sub population in west off Sumatra and another one is sub population in south of Java and Nusa Tenggara waters. The most of bigeye tuna catch (about 39%) was categorized as immature fish with Gonad Somatic Index stage I. The immature fish was mostly found in west off Sumatra waters. While the catch in south off Java and Nusa Tenggara waters was mostly categorized as mature fish. It was indicated that south off Java and Nusa Tenggara waters was as spawning ground of the big eye tuna.

Key words: Population structure, Big eye tuna, Indian Ocean, histology, genetic

INTRODUCTION

Big eye tuna (*Thunnus obesus*) is the second biggest tuna resource behind madidihang (*Thunnus alalunga*) in Indian Ocean. This species is predicted to live in all around the Indian Ocean and categorized as highly migratory species. The fish migrates accross oceans. North part of the Indian Ocean, stretches from west off Sumatra to south off Java-Nusa Tenggara, belongs to Indonesia. This area is very important for the tuna management purpose because south off Java-Nusa Tenggara is the most potential spawning ground for the big eye tuna resource. In order to manage properly this kind of fish both regional and international, accurate information about population structure and reproduction is needed.

Data about status of fish stock such as population structure, and reproduction become more important related to the big eye tuna resource in relation with other fish stocks in other places. In addition, increase level of exploitation needs to be monitored properly in order to identify development and changed of biomass. Availability of these data is usefull for Indonesia to actively contribute for international management of tuna stock as a unity especially in the Indian Ocean in the form of international organization of tuna (IOTC).

This paper will discuss population structure and reproduction of the big eye tuna resource in west off Sumatra and south off Java and Nusa Tenggara waters of Indian Ocean. It hopes that the discussion can be used as an important information for management purpose and future stock assessment.

MATERIAL AND PROCEDURE

Primary data collected was genetic base (mtDNA) obtained from histology genetic analysis of piece of fin and biology reproduction (*maturity* and *Gonado Somatic Index*). Data were collected by on board observation during 2010 in Hindian Ocean (Table 1 and Figure 1).

Table 1. Coordinates and Range of Size of Fish Taken From Each Group

Group	Coordinates		Size of fish (cm)
I	110.150°-120.475° E	8.962°-15.035° S	60-177
II	97.209°-100.503° E	2.035°-3.263° S	50-175

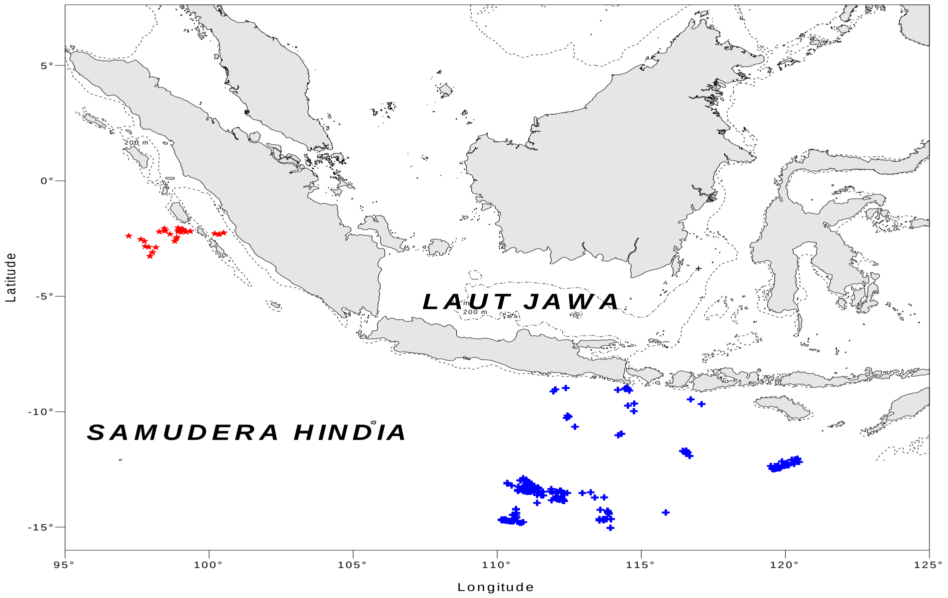

Figure 1. Sampling Site for genetic and gonad observation.

Sample of DNA was taken from histology of piece of fin using *Wizard Genome DNA Purification* kit (Promega). Primer Pro-5 and primer 12SAR were used to amplify sequence of mitokondria *D-loop* using *Polymerize Chain Reaction machine (PCR).* Sequence of mtDNA obtained was restricted using *endonuklease* based on enterprise standard procedure. Result of restriction was then separated through electrophoresis process using gel agarose 2-3% in *Tris-Boric-EDTA* (TBE) *buffer*. Product of restriction was identified using *UV illuminator* and printed out using polaroid film (Bremer, 1998).

Gonade preparation was done through HE (*Haematoxylin* and *Eoxin*) coloring technique toward piece of gonade histology sized 1 x 1 cm. *Histological maturity stage* was decided based on proportion of development of each *oocyte*.

Composite of haplotipe was analysed in order to define genetic parameter, population structure and phylogenic relationship each population. Genetic diversity degree was counted based on diversity index of haplotipe (*h*) through frequency distribution of haplotipe (nukleomorf) with equation as follow:

$$h = \frac{n}{n-1}(1 - \sum_{i=1}^{n} X_i^2)$$

Where : h = haplotipe diversity
n = number of group
X_i = frequency of haplotipe sample -i

- Genetic relationship of each population was decided based on genetic distance parameter (Nei, 1972) and statistic analysis of different restriction cite. The genetic distance was calculated according to equation introduced by Nei (1978):

$$D = -\ln[\frac{J_{ab}}{\{(J_a x J_b)^{0,5}\}}]$$

Where : D = Genetic distance
J_{ab} = Frequency of haplotipe of each place with same population.
J_a & J_b = Frekuency of haplotipe population A and B

- Degree differences of moleculer haplotipe of each population was predicted by means of *Analysis of Moleculer Varians* (AMOVA) and trial mixed distance (*Fst*) with equation as follow:

$$F_{st} = 1 - (\frac{H_w}{H_b})$$

Where : F_{st} = Diferential index
H_w = Average difference of intra population

H_b = Average difference of among population

- Phylogenic relationship among population was illustrated in the form of *clustering* dendrogram of mark of genetic distance based on average distance method.

Calculation was done using *Tools for Population Genetics Analysis* (TFPGA) introduced by Miller (1997).

Fish maturity was decided based on *Gonado Somatic Index* (GSI) equation introduced by Effendie (1997):

$$GSI= \frac{Wg}{W} \, x\ 100\%$$

Where W = Weight of fish (gram);
Wg = Weight of gonade (gram).

RESULT

1. Genetic Diversity and Population Structure of Big Eye Tuna

D-Loop mtDNA amplification of the fish using primer Pro-5 and primer 12SAR was noted as CAC GAC GTT GTA AAA CGA CCT ACC YCY AAC TCC CAA AGC and GGA TAA CAA TTT CAC ACA GGG CAT AGT GGG GTA TCT AAT CC respectively. This test resulted fragment DNA sizing about 1.500 bp (*base pairs*) in all groups of sample (Figure 1).

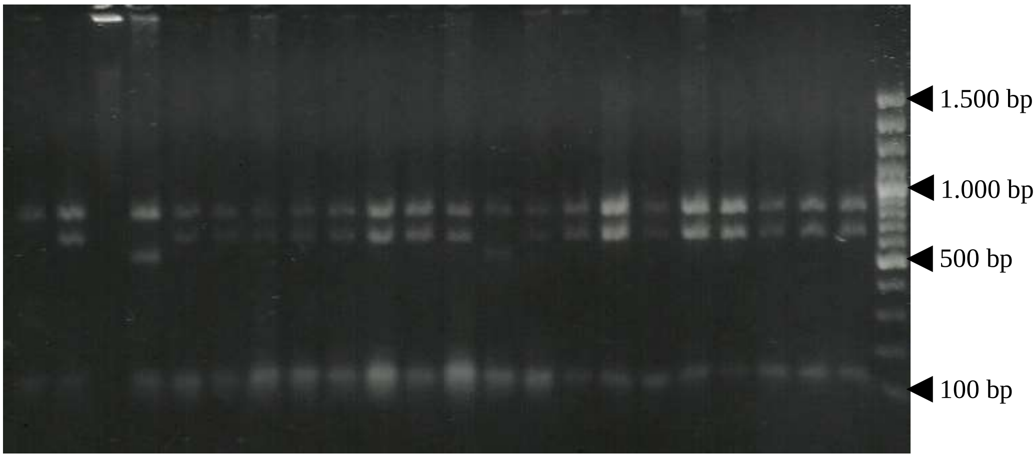

Figure 1. Single Fragment of mtDNA Tested by PCR amplification of Big Eye Tuna (*Thunnus obesus*) in Indian Ocean.

Diversity number and size of restriction fragment (RFLP) obtained from mtDNA restriction using four enzymes was 12 types of restriction namely *Taq I* with six types of restriction A, B, C, D, E, and F, *Hin6 I* with one type of restriction A, *Mbo I* with two types of restriction A, and B, and Rsa I with three types of restriction A,B, and C.

1.1. ***Haplotype Diversity***

Cutting of PCR product using four restriction enzymes resulted 12 composites of haplotype mt DNA *D-Loop region* where 10 composites were located in group I and eight composites were taken place in group II (Table 2).

Composite haplotype AAAA, AAAB, AAAC, AACA, ABAB and AAAD were distributed to all groups. All six composite haplotypes were catogorized as *major composite haplotypes* because they were taken place in both groups. Composite haplotype AABA, AACB, AABB and ABAA were only distributed in group I while composite haplotype AAAE and AAAF were only found in group II. These six composite haplotypes were categorized as *common haplotype* because they were only recorded in each group. In addition, composite haplotipe AAAB was the highest composite haplotype found in group II and group I as much as 36% and 29% respectively. In contrast, composite haplotype AAAA was identified in group II and group I as much as 29% and 26% respectively.

Mark of haplotype diversity of group I and group II was 0.7766 and 0.8267 respectively with an average of 0.8017 (Table 2).

Table 2. Haplotype frequency of mt-DNA *D-loop region* of big eye tuna in Indian Ocean Restricted using Four Enzymes *Taq I, Hin6 I, Mbo I* and *Rsa I.*

No	Type of composite haplotype	Frequency (%)	
		Group I	Group II
1	AAAA	0.26	0.29
2	AABA	0.08	-
3	AAAB	0.29	0.36
4	AAAC	0.03	0.04
5	AACA	0.03	0.04
6	AACB	0.03	-
7	AABB	0.10	-
8	ABAB	0.05	0.11
9	ABAA	0.05	-
10	AAAD	0.08	0.11
11	AAAE	-	0.04

12	AAAF	-	0.04
Amount of composite haplotype		10	8
Haplotype Diversity		0.8267	0.7766

1.2. Genetic Gap

Based on comparison test of *Fst* among groups using TFPGA program, it what found that there was significantly different between group I and group II (Table 3).

Table 3. Pair test (*Fst*) among group of sample of big eye tuna mata besar in Indian Ocean

	Group I	Group II
Group I	xxxxxxxxx	0.0212*
Group II		xxxxxxxxx

Note : *= Significantly different with $p<0.05$

Genetic gap and relationship among group of samples (phylogeny) of big eye tuna in Indian Ocean was 0.0038 (Table 4 and Figure 2)

Table 4. Genetic gap among group of samples (phylogeny) of big eye tuna in Indian Ocean

	Group I	Group II
Group I	xxxxxxxxx	0,0038
Group II		xxxxxxxxx

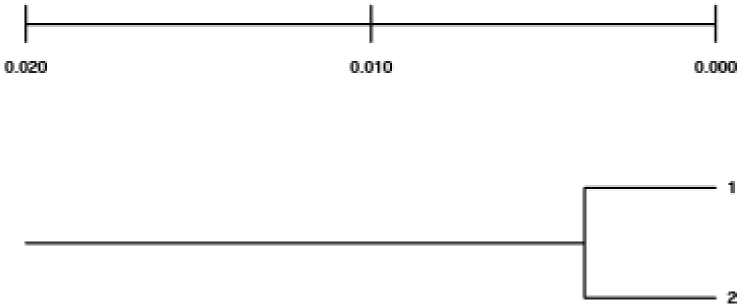

Figure 2. **Dendrogram of genetic relationship (phylogeny) of two different population of big eye tuna in Indian Ocean.**

1.3. Population Structure

Dendrogram illustration based on genetic gap showed different number among populations. This informed that the fish consisted of two sub populations, namely south off Java and Nusa Tenggara and west off Sumatra (Figure 3).

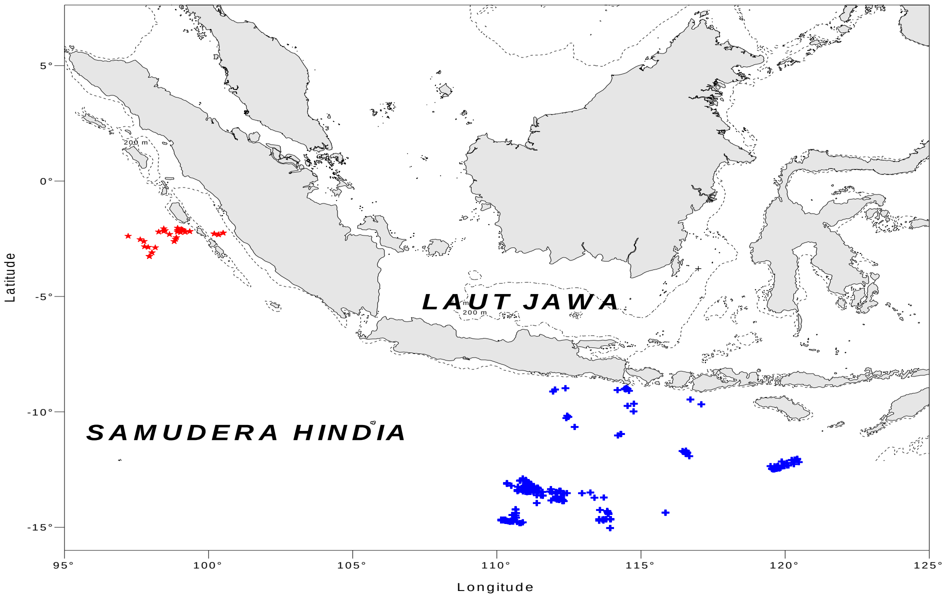

Figure 3. Population Structure of big eye tuna in Indian Ocean based on their genetic gap.

2. Reproduction of Big Eye Tuna

2.1. Gonade Maturity Level

Like reproduction of other vertebrata, edgg and spermatozoa of big eye tuna are separately formed. The fish has pairs of gonade, lobus left and right with almost same size. Based on histology assessment indicated that the fish was dominated by maturity level I accounted for 39%. This was followed respectively by level II (21%), III (17%), IV (11%). VI (5%), VII (4%), and V (3%) (Figure 4).

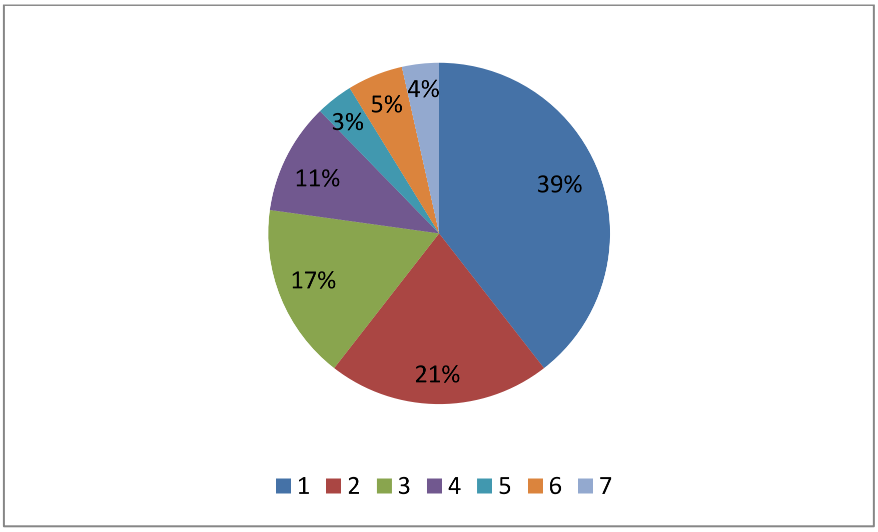

Figure 4. Percentage of Gonade Maturity Level of Big Eye Tuna in Hindian Ocean Based on Histology Identification.

In order to predict their spawning season, Level of gonade maturity must be compared to time of sampling. Figure 4 illustrated that percentage of each level increased. The fish with gonade maturity level I from April to October increased from 4.4% to 77.8%. Level II was found in April and October with frequency 8.3% and 91.7%. While the fish in level III was caught in June and October with frequency 5.3% and 94.7%. In contrast, level IV and level VII was only recorded in October and March respectively. In addition, the fish both level V and level VI were found in March, April, and October.

According to class of length, level I was found almost in all class of length except class 155-160 cm. In addition, the fish in level I was dominantly found in class 131-140 cm. On the other hand, level II had interval length of 91-150 cm and mostly found in class 101-110 cm, 121-130 cm, and 141-150 cm.. The fish with level III was identified in length of class 91-100 cm and 111-150 cm with the highest percentage in class 131-140 cm. Level IV was distributed in interval 101-150 cm with the highest percentage in class 111-120 cm. Different shape was showed by gonade maturity level

V. The fish in this level was only found in class interval 111-120 cm and 131-140 cm with the highest percentage in the later interval. Last but not least, level VI had three class interval, namely 91-100 cm, 121-130 cm, and 151-170 cm with the highest percentage in the middle interval. Finally, gonade maturity level VII was only found in class interval 121-140 cm with the highest percentage in interval 121-130 cm.

Figure 5 illustrated that the big eye tuna in maturity level I-III was caught in south off Java and Bali. While the fish in maturity level IV-VII was found in south off Nusa Tenggara. However the fish in maturity level III-V was also recorded in south of Java and Bali and the fish in maturity level II was identified in south off Nusa Tenggara.

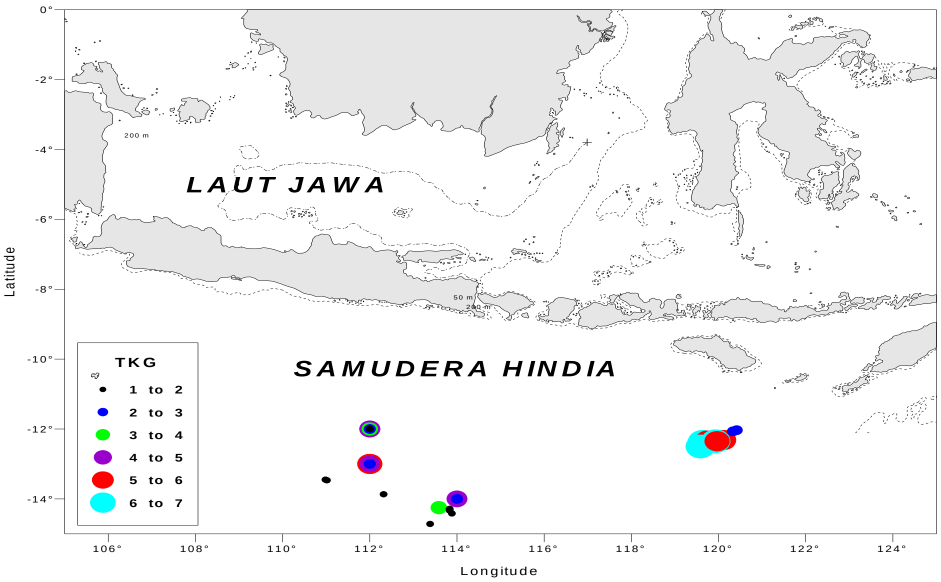

Figure 5. Fishing Ground of each maturity level of big eye tuna in Indian Ocean.

2.2 Gonada Somatic Index (GSI)

In general, mark of GSI increased and reached the peak when the fish spawned. Mark of GSI in march was 2.78 and decreased to 0.44 during April to June. In addition, the mark was going to increase to 0.47 and 0.86 in July and October respectively.

Trend mark of GSI was in line with maturity level of the fish. In maturity level I, mark of GSI was very low (0.51). While in maturity level II the mark increased to 0.71. The mark continued to increase in maturity level III, IV, V, VI, and reached the peak to be 3.19 in maturity level VII.

DISCUSSION

1. Genetic Diversity and Population Structure of Big Eye Tuna

The use of four restriction enzymes in this research was to identify variability of genetic diversity of population. Haplotype differences among and intra population was caused by changed, additional, and absence of certain chemical notation of *D-Loop region* mtDNA so that certain enzyme did not cut in the same location. This condition could cause the move of haplotype which indicated better genetic diversity among and intra population.

Genetic diversity level shown by number and diversity of haplotype of the big eye (0.8267 in group of sample I and 0.7766 in sample II) was equal with number of haplotype of other fish which accounted for 6 to 17 with diversity mark from 0.6 to 0.9 (Nugroho, 2001). Meanwhile Bremer *et al*., (1998) found that the big eye in Atlantic Ocean, Hindian Ocean, and Pacific Ocean had 13 composite haplotypes. Yet in Indian Ocean itself, the fish had about five haplotype composite.

Average mark of genetic diversity was 0.8017. This was a bit lower than average mark of yellow fin tuna, 0.857 (Permana *et al*., 2007) but a bit higher than other fast swimmer fish such as shark (0.64). (Heist, 1999 cited by Permana *et al*., 2007). Graves and McDowell (1994) cited by Tabata *et al*., (1997) illustrated that overall mark of haplotype diversity of all mtDNA of *stripead marlin* was 0.82. While Avise *et al*., (1989) cited by Tabata *et al*., (1997) added that haplotype diversity of all mtDNA of several fish ranged from 0.473 to 0.998.

This high mark of haplotype diversity indicated that the big eye tuna population in Indian Ocean especially group of sample I in south off Java and Nusa Tenggara was categorized as low disturbance stock. It also informed that the stock had higher level of migration habid than other species so that the stock had high chance to get cross spawning with other stocks (Wild, 1994 cited by Permana *et al*., 2007). In contrast, the fish in west off Sumatra with lower mark of haplotype diversity (0.7766) indicated that the stock had smaller size than another. Leary *et al*., (1985) cited by Ayu (2005) stated that lower mark of genetic diversity would cause negative impact such as the decrease of growth, size diversity, organ development stability, degree of life, and environment adaptation.

Genetic gap among big eye population in Indian Ocean was only 0.0038. The lower the mark of genetic gap the closer the fish diversity will be and vice versa. The big eye tuna in sample I (south off Java and Nusa Tenggara) had very low mark of genetic gap with the fish in sample II (west off Sumatra) (Table 4 and Figure 5). This phenomenon showed the closer relationship between them. It was predicted that fishing ground of both samples was not separated each other. Therefore migration and genetic change between them was often taken place. In addition, the big eye tuna had relatively equal mark of genetic gap with fish from same sub species such as king fish (Nugroho *et al*., 2001). Dendrogram based on genetic diversity illustrated that the stock of big eye tuna in Indian Ocean consisted of two sub populations, namely south off Java and Nusa Tenggara and west off Sumatra.

The occurrence of two different sub populations of big eye tuna in Indian Ocean was predicted to be caused by the difference of original region of both sub populations where they come from, namely Pacific Ocean and Indian Ocean. Sub population in west of Sumatra may have been the original stock while sub population in south off Java and Nusa Tenggara may have come from Pacific Ocean. Suda (1971) cited by Sumadhiharga (2001) stated that big eye tuna from Pacific Ocean often migrated to Indian Ocean through the Philippines and Indonesia waters.

Analysis toward both sub populations showed that they had high mark of genetic diversity. This indicated that the stock did not get too much disturbance. Better management is needed in order to keep the stock away from any disturbance so that the decrease of genetic diversity can be avoided.

2. Reproduction of Big Eye Tuna

Size of big eye tuna ranged from 60 to 177 cm with modus in interval 90-99 cm. Most of samples (about 90%) was categorized as matured fish. Nootmorn (2004) reported that size at first maturity of big eye tuna in Indian Ocean for female and male was 88.08 cm and 86.85 cm respectively.

Gonade maturity is the most important stage in development of fish. During reproduction, most energy is spent to develop gonade in maturity stage. Weight of gonade will reach maximum just before fish get to spawn and then the weight will go down quickly during spawning process until finish. Effendie (1997) stated that gonade weight for female and male could reach 10-25% and 5-10% of body weight respectively. He added that the higher the level of gonade maturity, the bigger the egg in gonade would be. Kuo *et al*., (1973) emphasized that level of gonade maturity was indicated by development of average diameter of egg and distribution type of egg size.

Based on gonade maturity level and size length of class informed that length of fish in maturity IV to VII ranged from 91 cm to 170 cm. Previous research stated that size of fish at first maturity (Lm) of big eye tuna in Indian Ocean was 88.08 cm (Nootmorn , 2004) and 102.4 cm (Farley *et al.*, 2003). another research informed that size of fish at first maturity of big eye tuna in Pacific Ocean was 91-100 cm in length and 14 – 20 kg in weight (Yuen, 1955). Sun *et al.*, (2006) added that big eye tuna in Pacific Ocean already matured as long as 99.7 cm.

Average mark of GSI went down in March to June. Relationship between gonade maturity level and GSI showed that average mark of GSI increased in line with with the increase of gonade maturity level. Effendi (1997) stated that comparison between body weight and gonade weight increased due to higher level of gonade maturity level. Mark of GSI of big eye tuna in Indian Ocean during March to October varied. The highest mark obtained in March. This meant that the fish spawned before March. Nootmorn (2004) gave different opinion. He found that spawning activity of fish in east part of Indian Ocean occured in December, January, and June. While in east part of Pacific Ocean, spawning activity of fish was taken place during April to September in north latitude and during January to March in south latitude.

Different result of the research compared to previous research was due to incomplete data obtained. The assessment of the research did not conduct all the year so that data about peak of spawning season ws not collected. Another reason was, as stated by Nishikawa (1985), that around equator in Atlantic, Indian, and Pacific Oceans the fish spawned during gthe year. Sun *et al.*, (2006) added that big eye tuna in east part of Pacific Ocean had spawning activity during April to September, January to March, and October to January.

CONCLUSSION

1) Population Structure of big eye tuna in Indian Ocean consisted of two different sub populations, namely west off Sumatra and south of Java and Nusa Tenggara.
2) It was predicted that sub population in south off Java and Nusa Tenggara come from Pacific Ocean while sub population in west off Sumatra was the original stock of Indian Ocean.
3) Big eye tuna was dominated by gonade maturity level I (39%) and the spawning season of big eye tuna in Indian Ocean occurred during all the year. With spawning ground located in south off Java-Nusa Tenggara.

REFERENCES

Ayu, H.S. 2005. Genetic diversity Evaluation of four population of carper (*Oreochromis niloticus*) based on mtDNA *D-loop* analysis[skripsi]. Fisheries Department, Faculty of Fisheries. Juanda University. Bogor

Bremer, J.R.A., B. Stequert, N.W. Robertson dan B. Ely. 1998. Genetic evidence for inter-oceanic subdivision of bigeye tuna (*Thunnus obesus*) populations. *Marine Biology* 132:547-557.

Effendie, M.I. 1997. Fisheries Biology. Yayasan Pustaka Nusantara.

Farley, J., Clear N., Leroy B., Davis T. dan Mcpherson G. 2003. Age and growth of bigeye tuna (*Thunnus obesus*) from the eastern and western AFZ. *Report No. 2000/100*. CSIRO Marine Research. Australia. 93p.

Kuo, C.M., Shehadeh Z.H. dan Milisen K.K. 1973. A preliminary report on the development, growth and survival of laboratory reared larvae of the grey mullet, *Mugil cephalus* L.J. *Fish. Biol*., 5:459-470.

Miller, M.P. 1997. *Tools for Population Genetic Analyses (TFPGA} version 1.3*. Department of Biological Sciences-Box 5640. Northern Arizona University

Nei, M. 1972. Genetic distance between populations. *American Nature* 106:283-292.

Nei, M. 1978. *Molecular Evolutionary Genetics*. New York: Columbia University Press.

Nishikawa, Y., Honma M., Ueyanagi S. dan Kikawa S. 1985. Average distribution of larvae of oceanic species of Scombroid fishes, 1956–1981. *S Ser.Far Seas Fish.Res.Lab.*, (12):99 p.

Nootmorn, P. 2004. *Reproductive Biology of Bigeye Tuna in the Eastern Indian Ocean*. Document IOTC-2004-WPTT-05, presented at the Working Party on Tropical Tunas, Victoria, Seychelles, July 13th-20th 2004.

Nugroho, E., Ferrel D.J., Smith P. dan Taniguchi N. 2001. Genetic divergence of Kingfish from Japan, Australia and New Zealand Inferred by microsatellite DNA and mitochondrial DNA control region markers. *Journal Fisheries Science* 67:843-850.

Permana, G.N., J.H. Hutapea, Haryanti dan S.B.M. Sembiring. 2007. Genetic Variety yellow fin tuna (*Thunnus albacares*) using electrophoresis Allozyme and Mt-DNA analysis. Aquaculture Research Journal 2(1):41-50.

Sumadhiharga, K. 2001. Tuna Fishery in Indonesia. Paper presented in Innaguaration of Primary Research Expert of Marine Biology in Oseanography Research Center Science Research Board of Indonesia, 6th September 2001.

Sun, C.L., Chu S.L. dan Yeh S.Z. 2006. The Reproductive Biology of Female Bieye Tuna (*Thunnus obesus*) in the Western Pacific. Scientific Committee Second Regular Session. Manila, Philippines. 22p.

Tabata, K. dan A. Mizuta. 1997. RFLP analysis of the mtDNA D-loop region in red sea bream, *Pagrus major* population from four locations of western Japan. *Journal Fisheries Science* 63(2):211-217.

Yuen, H.S.H. 1955. Maturity and fecundity of bigeye tuna in The Pacific. U.S. Fish Wildl. Serv., *Spec.Sci.Rept.Fish.,* 150:30p.