

# Genetic Diversity and Demography of Skipjack Tuna (*Katsuwonus pelamis*) In Southern and Western Part of Indonesian Waters

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## Abstract

Skipjack tuna (*Katsuwonus pelamis*) is highly migratory species that spread from trophic and sub trophic waters. This species can be found in Atlantic, Indian and Pacific oceans. The genetic information of highly migratory species like skipjack tuna is important to support the sustainability of the fisheries. The objectives of this study are to gain information genetic diversity and population structure of exploited species and to understand the population kinship in Indonesian waters. Tissue samples were collected from six locations, i.e.: Sibolga (North Sumatera), Padang (West Sumatera), Binuangeun (Banten), Pacitan (East Java), Lombok (West Nusa Tenggara) and Kupang (East Nusa Tenggara). Microsatellite analysis was done in this study consisting of extraction, purification, polymerase chain reaction (PCR) amplification and electrophoresis. Three loci used for the analysis i.e.: UTD 172, UTD 523 and UTD 535. The results showed that there are two groups from six locations i.e.: group 1: Sibolga and Padang; group 2: Binuangeun, Pacitan Lombok and Kupang. The variance among these two groups is 0.066 with variance 5.441%. This finding in line with Indonesian Fisheries Management Area of 572 (west of Sumatera waters) and 573 (south of Java waters). However, as highly migratory species across nations, the management strategy for skipjack tuna needs collaboration among countries through regional fisheries management authority like Indian Ocean Tuna Commission (IOTC).

**Keywords:** population structure, Tuna fish, microsatellite analysis

## Introduction

Skipjack tuna (*Katsuwonus pelamis*) is a cosmopolitan pelagic fish and could be found in with in both tropical and subtropical waters of the three major oceans, e.g. Indian, Pacific and Atlantic Oceans (Fujino *et al.*, 1981). It is highly productive (Grande *et al.*, 2014) with length at maturity 43 cm (Jatmiko *et al.*, 2015) and has a maximum age below 4.5 y (Jin *et al.*, 2015). It is also considered as an important fisheries commodity in Indonesia, especially in small-scale fisheries (Setiyawan, 2016; Nurdin and Nugraha, 2017; Setyadji *et al.*, 2018). Based on Indian Ocean Tuna Commission (IOTC) database, the exploitation of skipjack by Indonesian fleets has been conducted since early 1950s. The catch estimation of skipjack is rising up from only ~500 tons in 1950 to ~92,000 tons in 2013. However, the catch is declining and reach stable posture around 76,000-78,000 tons during 2014-2016 (IOTC, 2018).

Skipjack interacted with various gears such as hand line, troll line, purse seine, gillnet, longline and pole and line. Therefore, it susceptible to local overfishing as intensive fishing occurred throughout the years, especially in western and southern part of Indonesian waters (Rochman *et al.*, 2015; Zedta *et*

*al.*, 2018). No specific stock assessment conducted yet for skipjack either in western or southern part of Indonesia, but the initiative has begun in its territorial and archipelagic waters (Satria and Sadiyah, 2018). Determining the stock distribution or origin is essential before conducting any stock assessment, because it will shape the most suitable harvest control rules.

Population structure in population genetics is explaining the management unit of the population (stock) of fish. Several approaches have been taken to explain the condition of fish population structure, including phylogeny, genetic relationships and genetic variation among populations (Jatmiko, *et al.*, 2018; Barth *et al.*, 2017; Iranawati, 2014). Previous genetic stock structure studies on skipjack revealed a close relationship between Atlantic and Pacific stock (Ely *et al.*, 2005; Graves *et al.*, 1984) but distinct with Indian Ocean stock (Fujino *et al.*, 1981; Menezes *et al.*, 2006). A few "localized" genetic study also conducted by Dammannagoda *et al.* (2011) in Northwestern Indian Ocean, which found two distinguishable stocks mixed in Sri Lankan waters. Similar studies were also conducted in Indonesia, e.g. Fakhri *et al.* (2015) revealed no differentiated population of skipjack from western and eastern part of Bali. A different approach was

used by (Wujdi *et al.*, 2017) in order to distinguish skipjack stock in south part of Indonesian waters using otolith shapes. The study concluded a single population throughout southern part of Indonesian waters. However, a much broader skipjack genetic stock structure study is required to fully understand about the possibility of any distinguishable stocks between western and southern part of Indonesian waters. The result hopefully can be a foundation of any stock assessment of skipjack tuna in the future.

**Material and Methods**

Samples of skipjack were collected from 6 landing sites. Two from western part of Sumatra, e.g. Sibolga (SB), Padang (PD), and the rest (4) from south of Java, Bali and Nusa Tenggara, e.g. Binuangun (BN), Pacitan (PC), Lombok (LO) and Kupang (KU) (Figure 1.) during July-August 2017. In order to reduce bias, only FAD's associated, fresh landed and small sized skipjack were chosen (<30 cm), under assumption that small fishes' movement is still limited. Total genomic DNA (20 samples from each location) was extracted from muscular body tissue (Table 1.) and stored in absolute alcohol.

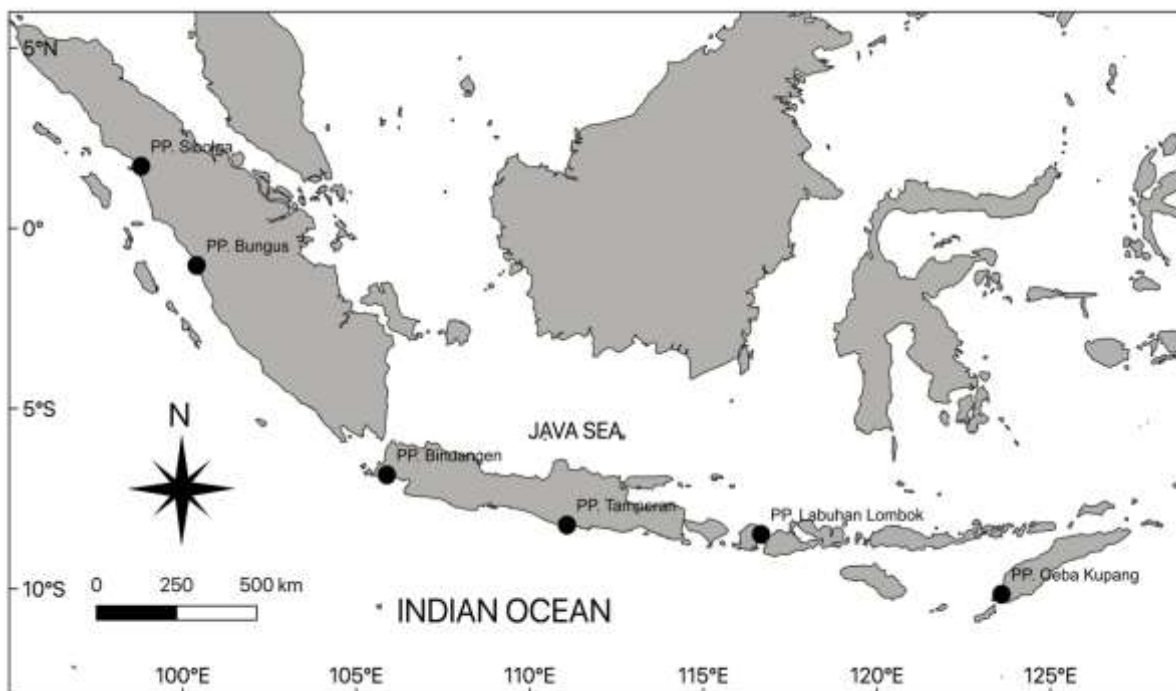
**DNA extraction**

Approximately every 0.2 mm of muscular tissue was transferred into an *ependorf* tube

contained 200 µl of 10% chelex solution in ddH<sub>2</sub>O and resin solution (Walsh *et al.*, 1991), it later mixed using vortex for 1 minute. The solution was centrifuged at 10,000 rpm for 1 minute using microcentrifuge and incubated for 40 minutes at 95°C using a heating block or thermo shaker. The sample was mixed again using vortex for 1 minute and centrifuged for 1 minute at 10,000 rpm. DNA samples in the form of supernatants were taken and stored in a refrigerator at -20°C until further use.

**PCR amplification**

Microsatellite markers used for this study referred to previous work by Dammannagoda *et al.* (2011). Three tetra-nucleotide microsatellite loci (UTD172, UTD535, and UTD523) were amplified and analyzed here. UTD172 (Forward: 5'-GTT GTG TAT TGG CTG GAC C-3 'TTT; Reverse: 5'-CAA CAG CT GGC AAA TTC CG-3'), UTD 523 (Forward: 5'-TTT GAA TGG GAG ACA TGC AG -3 '; Reverse: 5'-TGT CCT GCA CTT GTG TTC ACT -3') and UTD 535 (Forward: 5'- CAC TGA AGA TAT AGG CAG CCT TG -3 '; Reverse: 5'- TTT CTC CAG CGG CAT TAC AT -3'). Microsatellite PCR reaction consisted of 10 µl HotStar<sup>®</sup> Master Mix, 2 µl sterilized water, 1 µl primer F, 1 µl primary R, 6 µl DNA template. PCR conditions were as follow: pre-denaturation phase (3 minutes at 94°C), denaturation (30 seconds at 94°C), primer attachment/annealing (30 seconds at 52°C), fragment lengthening/extension (1 minute at 72°C).



**Figure 1.** Skipjack tuna (*K. pelamis*) sampling sites in Southern and Western Part of Indonesian Waters

All cycles were repeated 34 times. In order to improve the result, the final elongation stage/final extension was carried out for 10 minutes at 72 °C and stored at room temperature (~24 °C) for 1 minute.

**Electrophoresis**

Electrophoresis was performed on 1.5% agarose gel in 10x TBE buffer, at 100 V, with electric current 400 mA for 30 minutes. The DNA fragments visualized using an ultraviolet (UV) transilluminator and documented with a digital camera using the UVITEC® device.

**Data analysis**

Analysis of DNA fragments was detected by the Applied Biosystems Genetic Analyzer® and then interpreted using the GeneMapper v4.0. This is required to clarify the interpretation of the DNA fragments' size (alleles) that appeared from each DNA genome. The 2-D look describe the allele's size (X-axis) and peak height (Y-axis) which showed the concentration of the results of the fragment analysis. The fragment analysis process was conducted at 1<sup>st</sup> BASE Laboratory in Singapore. Each locus and each site were tested for deviation from Hardy-Weinberg equilibrium (HWE) in poppr package (Kamvar et al., 2014; Kamvar et al., 2015) under R version 3.5.0 (R Core Team, 2018) with significance of deviations in observed vs. expected heterozygosity tested using Chi-square. DNA diversity (molecular diversity indices), inbreeding coefficient ( $F_{IS}$ ) for each locus and each site and differentiation in population which determined the stock structure were tested using Arlequin v3.5 (Excoffier and Lischer, 2010). Analysis of Molecular Variance (AMOVA) was performed to determine the genetic variation and population structure among the skipjack population group.

**Results and Discussion**

**Genetic variability and Hardy-Weinberg equilibrium**

Genetic analyses were conducted on 120 individuals sampled from Sumatra (Sibolga, SB; Padang, PD), Java (Binuangeun, BN; Pacitan, PC), Nusa Tenggara Barat (Lombok, LO) and Nusa Tenggara Timur (Kupang, KU) (Figure 1). No null alleles, a total of 353 alleles were produced from 3 microsatellite loci across 6 sampling sites, with average abundance 23.56/site. Since all the data was diploid and the number of samples every location is similar, hence, number of multilocus genotype (MLG), diversity index ( $h$ ) and evenness index ( $E_5$ ) resulted the same (Table 1.). Sample populations were then tested for conformation to HWE. Both the analytical p-value and permuted p-value showed enough confidence that all loci are not under the null expectation of HWE ( $P > 0.001$ ). Therefore, all three loci were therefore included in all further analyses. The genetic diversity of each group/population was represented by the value of heterozygosity/expected heterozygosity ( $H_e$ ). The lowest mean abundance value of  $H_e$  was found in Binuangen (0.6735), as the highest was in Sibolga (0.8557) (Table 2.).

**Population structure**

Pairwise distance test between the population ( $F_{ST}$ ) and AMOVA were performed in order to investigate whether there are any differences in skipjack population structure.  $F_{ST}$  value among the sample groups in all microsatellite loci were calculated under 99.95% confidence interval ( $P < 0.05$ ). Pairwise  $F_{ST}$  analysis of microsatellite data identified three different group of populations, PD and SB, BN and PC, LO, and KU (Table 3.).

**Table 1.** Sampling details and descriptive microsatellite statistics for skipjack tuna samples

Population	<i>n</i>	MLG	eMLG	se	<i>h</i>	<i>G</i>	<i>lambda</i>	<i>E.5</i>	<i>Hexp</i>	<i>Ia</i>	<i>rbarD</i>
Lombok	20	20	20	0	3	20	0.95	1	0.781	-0.0159	-0.00884
Padang	20	20	20	0	3	20	0.95	1	0.823	0.0223	0.02263
Sibolga	20	20	20	0	3	20	0.95	1	0.845	-0.2119	-0.11591
Binuangen	20	20	20	0	3	20	0.95	1	0.664	-0.1084	-0.05442
Pacitan	20	20	20	0	3	20	0.95	1	0.755	0.0793	0.04815
Kupang	20	20	20	0	3	20	0.95	1	0.75	-0.1298	-0.06905
Total	120	120	20	0	4.79	120	0.992	1	0.794	0.0284	0.01507

**Note:** Variables are represented by the following: *n*, sample size; MLG, Number of multilocus genotypes (MLG) observed; eMLG, The number of expected MLG at the smallest sample size  $\geq 10$  based on rarefaction; se, standard error based on eMLG; *h*, Shannon-Wiener Index of MLG diversity (Shannon, 1948); *G*, Stoddart and Taylor's Index of MLG diversity (Stoddart & Taylor, 1988); *lambda*, Simpson's Index (Simpson, 1949); *E.5*, Evenness Index,  $E_5$  (Grunwald et al., 2003; Ludwig and Reynolds, 1988; Pielou, 1976); *Hexp*, Nei's unbiased gene diversity (Nei, 1978); *Ia*, The index of association (Brown et al., 1980; Smith et al., 1993); *rbarD*, The standardized index of association.

**Table 2.** Descriptive statistics for three microsatellite loci among skipjack collections

Location		Locus			Mean across loci
		UTD 172	UTD 523	UTD 535	
Padang (PD)	<i>n</i>	20	20	20	20
	<i>a</i>	2	30	40	24
	<i>H<sub>e</sub></i>	0.5128	0.9807	1.0000	0.8312
	<i>F<sub>IS</sub></i>	-1.0000	-0.0201	-0.0201	-0.3467
Sibolga (SB)	<i>n</i>	20	20	20	20
	<i>a</i>	4	26	38	22.66
	<i>H<sub>e</sub></i>	0.5923	0.9780	0.9960	0.8557
	<i>F<sub>IS</sub></i>	-0.6298	-0.0224	-0.0228	-0.6750
Binuangeun (BN)	<i>n</i>	20	20	20	20
	<i>a</i>	2	25	38	21.66
	<i>H<sub>e</sub></i>	0.5000	0.9731	0.9974	0.6735
	<i>F<sub>IS</sub></i>	-0.0000	-0.0284	-0.0242	-0.3912
Pacitan (PC)	<i>n</i>	20	20	20	20
	<i>a</i>	6	28	40	24.66
	<i>H<sub>e</sub></i>	0.3205	0.9769	1.0000	0.7658
	<i>F<sub>IS</sub></i>	0.0656	-0.0243	-0.0256	0.0052
Lombok (LO)	<i>n</i>	20	20	20	20
	<i>a</i>	5	26	39	23.33
	<i>H<sub>e</sub></i>	0.3846	0.9795	0.9987	0.7876
	<i>F<sub>IS</sub></i>	-0.1753	-0.0215	-0.0201	-0.0723
Kupang	<i>n</i>	20	20	20	20
	<i>a</i>	4	26	40	23.33
	<i>H<sub>e</sub></i>	0.3141	0.9756	1.0000	0.7633
	<i>F<sub>IS</sub></i>	-0.1176	-0.0256	-0.0284	0.0573

Note: *n*-number of samples, *a*-number of alleles, *H<sub>e</sub>*-expected heterozygosity and *F<sub>IS</sub>*-inbreeding coefficient.

**Table 3.** Microsatellite pairwise *F<sub>ST</sub>* value among the sampling locations

Population	Padang	Sibolga	Binuangeun	Pacitan	Lombok	Kupang
Padang	-	0.4144	0,0001**	0,0001**	0,0001**	0,0001**
Sibolga	0.0000	-	0,0001**	0,0001**	0,0022**	0,0001**
Binuangeun	0.0956	0.0723	-	0,0180*	0,0006**	0,0090**
Pacitan	0.0696	0.0455	0.0122	-	0,1801	0,1803
Lombok	0.0335	0.0166	0.0158	0.0032	-	0,1982
Kupang	0.0700	0.0476	0.0103	0.0031	0.0032	-

Note: \* *P*<0.05; \*\* *P*<0.01

**Table 4.** Population structure based on differences in genetic variation of skipjack populations (AMOVA)

No.	Group simulations	Variance among groups	% variance	FCT	<i>p</i> value
1	(PD,SB); (BN,PC,LO,KU)	0,066	5,441	0,054	0,016**
2	(PD,SB); (BN,PC); (LO,KU)	0,045	3,804	0,038	0,039**
3	(PD,SB); BN; PC; (LO,KU)	0,046	3,866	0,039	0,010***
4	(PD,SB); BN; PC; LO; KU	0,044	3,893	0,037	0,021**

Note: \*\* *P*<0.05; \*\*\* *P*<0.01

The result then simulated into various group and analyzed with AMOVA. Among 4 group simulated, significant differentiation was evident among populations within locations. Group number 1 (PD,

SB and BN, PC, LO, KU) showed the highest variance among other groups (Table 4.). Therefore, rather than divided into 3 populations as a result from microsatellite pairwise, we chose the output from

AMOVA analysis, which the populations were separated into two main group.

By far, the skipjack in Indian Ocean are currently managed as a single stock, yet previous and recent study strongly suggested some mixed stocks exist in Sri Lankan waters (Dammannagoda *et al.*, 2011) and southern part of Indonesian waters. Intensive fishing pressure on particular fish stock known could cause population subdivision, lower genetic variation and produce selective genetic alteration (Allendorf *et al.*, 2008). Latest report shown that total catches in 2017 (524,282 t) were 10% higher than the catch limit generated by the Harvest Control Rule (470,029 t) (IOTC, 2018). Although no sign of overfished or overfishing in the process. But it could be one of the intrinsic factors related to stock separation in Indian Ocean.

Most of skipjack fishing are assisted with FADs, especially in coastal areas (Davies *et al.*, 2014; Marsac *et al.*, 2017; Rodriguez-Tress *et al.*, 2017). Since the successful implementation of anchored FAD for commercial purpose in 1985 (Yusfiandayani *et al.*, 2015), it has been used intensively in western part of Sumatra and south of Java waters. Skipjack known to travel long and fast, for instance, based on recent tagging study, it could reach average distance of 800 km in just a month after release (Fonteneau, 2014). But, the long use of FAD could alter the behavior of skipjack, the present of 4-5 FAD in 50x50 km area could hold the movement of skipjack up to 50% (Kleiber and Hampton, 2011) although not for a long period of time (Govinden *et al.*, 2013). A theory developed by Marsac *et al.* (2000), and later discussed by Hallier and Gaertner (2008) suggested, that both type of FADs (drifting or anchored) are acting as a super-stimulus, which misleading tunas to make inappropriate habitat selection or mostly known as ecological trap.

In this study, pairwise distance test suggested 3 distinguishable population of skipjack, 1 population in the west, and 2 separate populations in south (Table 2.). However, further analysis using AMOVA suggested only 2 distinguishable populations based on the highest variance percentage among group scenarios. The later result was more reasonable since there is no geographical barrier between two sites and located relatively close distance. The rough distance between BN and PR is around 555 km, a reachable distance for skipjack based on tagging study (Fonteneau, 2014). Moreover, most of the genetic samples were derived from troll and handline fisheries, which their fishing grounds are located inside the EEZ. So, there is no strong motivation to distinguish the population of skipjack in southern of Indonesia to more than one.

Allele size distribution in this study is similar with (Dammannagoda *et al.*, 2011), most likely due identical markers used. Loci UTD-523 and UTD-535 were highly polymorphic across skipjack samples, while loci UTD-172 was less polymorphic. Genetic polymorphism promotes diversity, and diversity means stability within population. Overall, skipjack population showed a high level of genetic diversity (mean  $H_e = 0.780$ ), despite area around western part of Sumatra and southern part of Jawa, Bali and Nusa Tenggara known as intensive fishing ground of skipjack. This condition occurred, perhaps due to continuous gene flow from other areas, such as western Indian Ocean and archipelagic waters (Banda Sea) (Natsir *et al.*, 2012; Fonteneau and Hallier, 2015). Higher allelic skipjack diversity is maintained by its large population throughout the world (Avice *et al.*, 1998; Chiang *et al.*, 2008), it also a typical of genetic pattern from Scombridae family (Zardoya *et al.*, 2004). However, among all locations, Binuangen has the lowest genetic diversity, the cause is still unclear, but localized depletion from both troll line/hand line and purse seine fleets might be the cause. It is understood that above-mentioned fleets are sharing the same fishing ground, around the FADs. Continuous harvesting from the same FADs without giving time to replenish the stock could provoke growth overfishing on skipjack. Naturally, during November to March the effort will drop due to bad weather condition triggered by monsoon season. During this time, skipjack will have enough time to rejuvenate themselves. But in Binuangen, the fishing happens all year despite the monsoon period, resulted in small skipjack caught during the particular period. An open close system perhaps is the viable solutions for both conservation and exploitation purpose. Maintaining high genetic diversity is key to sustainability. Populations with high genetic diversity have a better chance of survival because each gene has a different response to environmental conditions. The presence of various genes from individuals in the population increases the ability of the population to respond to environmental changes (Wright, 2005).

## Conclusion

This study reveals that there are two stocks of skipjack tuna in Indonesian waters, in Indian Ocean area, i.e. west of Sumatera waters and south of Java waters. This finding in line with Indonesian Fisheries Management Area of 572 (west of Sumatera waters) and 573 (south of Java waters). However, as highly migratory species across nations, the management strategy for skipjack tuna needs collaboration among countries through regional fisheries management authority like IOTC.

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