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Short Communication: Genetic structure of Longtail Tuna *Thunnus tonggol* (Bleeker, 1851) in Java Sea, Indonesia

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Abstract. *Al Malik MD, Pertiwi NPD, Sembiring A, Yusmalinda NLA, Ningsing EY, Astarini IA. 2020. Short Communication: Genetic structure of Longtail Tuna Thunnus tonggol (Bleeker, 1851) in Java Sea, Indonesia. Biodiversitas 21: 3637-3643. Thunnus tonggol* (Longtail Tuna) is an economically important fish found in Indonesia waters, however, the information regarding this fish is lacking. Known to be a neritic fish and found in shallow water, Java Sea is one of the ideal habitats for *T. tonggol* species. Due to high fishing rates activities in Java Sea, a better management plan to ensure the conservation and fisheries sustainability around this area is needed, especially to protect *T. tonggol* population. In order to complete the Indonesian tuna data, we aim to study the diversity and genetic structure of *T. tonggol* in Java Sea at three different locations; i.e. Semarang, Banjarmasin, and Jakarta. In this study, population genetic methods with the marker of mitochondrial DNA (mtDNA) control region were used in population structure analysis. A total of 115 specimens were collected from the fish market around the area of study locations and amplified using *polymerase chain reaction* (PCR) and sequenced using Sanger methods. The result showed genetic diversity (Hd) value of 0.99366, and nucleotide diversity (π) value of 0.01906. Both of these values indicated high genetic diversity. Population analyses using Analysis of Molecular Variance (AMOVA) showed nonsignificant differences between the three populations of study (mixing population), with the Φ_{ST} value of 0,00375 (p-value > 0.05). Based on this result, the fisheries management for *T. tonggol* in Java Sea needs to be managed as one single population management.

Keywords: Connectivity, control region, genetic, fisheries management, Longtail Tuna

INTRODUCTION

Tuna is one of the important fisheries in the world (Kunal et al. 2013; Benetti et al. 2016) and known as a vital commodity in Indonesia, such as *Katsuwonus pelamis* (Skipjack Tuna), *Thunnus obesus* (Bigeye Tuna), and *Thunnus albacares* (Yellowfin Tuna) (Sunoko and Huang 2014; Suhana et al. 2016). Total production tuna in the world has reached 7.9 million tones, and Indonesia has reached 6.71% of the total marine capture production, including tuna (FAO 2020a,b). Indonesian local tuna fisheries have been mainly on the small-scale and artisanal fisheries which targeting the neritic tuna (Amri and Satria 2013; Babu and Anrose 2013).

Longtail Tuna (*Thunnus tonggol*) is a neritic tuna with the same group as Frigate Tuna (*Auxis thazard*), Kawakawa (*Euthynnus affinis*), and Skipjack Tuna (*K. pelamis*) that found mostly near coastal areas (Yesaki 1994; Griffiths et al. 2009; Abdussamad et al. 2012). Longtail Tuna (*T. tonggol*) has been known as one of the important tuna species for artisanal fisheries (Sadough et al. 2018). This type of tuna usually caught using fishing tools such as gillnets, seine nets, and trolling (Sharma et al. 2012; Restiangsih and Hidayat 2018). The total world production of Longtail Tuna has reached 237. 124 tonnes in 2016 (FAO 2020b), meanwhile in Indian Ocean, including Indonesia, the annual catches reach 116.000 tons (Abdussamad et al. 2012).

Longtail Tuna usually found around Indo-Pacific waters (Sharma et al 2012; Kunal et al 2014; Froese and Pauly 2019). In Indonesia, *T. tonggol* species is easy to find within the area of Java Sea, including in the Fisheries Management Area-712 (FMA-712) with a total area of 320.000 km^2 (Restianingsih and Hidayat 2018); Kep-Men KKP 2016). In their report, it is also mentioned that there is indication of full exploitation of Longtail Tuna population within Java Sea (Restianingsih and Hidayat 2018), which will impact the sustainable fisheries of this species. Furthermore, a study by Sharma et al. (2012), showed that the status of Longtail Tuna in Indo-Pacific has been approaching overfishing category. Although it is reported that Indonesia's Longtail Tuna fisheries have reached about 29% of all caught in Indian Ocean, and placed at the second top after Iran (42%), the stock status of Longtail Tuna is uncertain due to the lack of data (Sharma et al. 2012).

Several studies of Longtail Tuna (*T. tonggol*) has been conducted by taking the total catch and total length on the landing sites (Abdussamad et al. 2012; Restianingsih and

Hidayat 2018); however, fisheries landing reports may not always represent the actual data, due to the species miss-identification thus lead to miss-calculation of the number of catch reports (Pauly and Froese 2012). Therefore, to add more information on this tuna landing reports, genetic studies were conducted. Previous genetic studies to understand the population structure of Longtail Tuna have been conducted in India (Kunal et al. 2014; Kumar et al. 2016) and South China Sea (Willette et al. 2016), but no report on genetic information of Longtail Tuna in Indonesia, particularly in Java Sea. This genetic information can also be used to infer the stock structure of the Longtail Tuna population within Java Sea, and also across Indonesia. Therefore, in this study, we aim to explore the population genetic structure of Longtail Tuna (*T. tonggol*) in Java Sea using molecular genetic, which is known as a powerful tool to study population. Understanding this population of *T. tonggol* in Java Sea could become an important data reference to help the conservation manager and policymaker in managing the sustainable fisheries of this species in the area of study (Pertiwi et al. 2017).

MATERIALS AND METHODS

Samples were collected from fish markets on three different locations including Jakarta (n= 19), Semarang (Central Java) (n= 38), and Banjarmasin (South Kalimantan) (n= 58) (Figure 1). Samples were collected in the form of fin clips or meat and preserved in 96% ethanol. Fishing ground location was also confirmed to the local fishermen to ensure the samples were taken on the area of Java Sea (FMA-712). Samples were collected in the period of 2018 until 2019. Samples were taken to laboratory on a cooler box with ice.

This research used a molecular genetic approach using the mitochondrial DNA (mtDNA) of Control Region (D-loop) locus. DNA extraction was done using 10% Chelex method (Walsh et al. 1991). Mitochondrial (mtDNA) control region fragment was amplified using *Polymerase Chain Reaction* (PCR), with forward primer (CRK: 5'-AGC TCA GCG CCA GAG CGC CGG TCT TGT AAA – 3') and reverse primer (CRE: 5' – CCT GAA GTA GGA ACC AGA TG – 3') (Lee et al. 1995).

Table 1. The value of genetic diversity of Longtail Tuna (*Thunnus tonggol*) based on three populations

Population	Hn	Hd	π	N
Semarang	32	0,99004	0,01932	38
Banjarmasin	49	0,99456	0,02414	58
Jakarta	15	0,97076	0,02174	19
All	85	0,99268	0,02217	115

Note: N = Number of sample, Hn = Number of Haplotype, Hd = Haplotype diversity, π = Nucleotida diversity

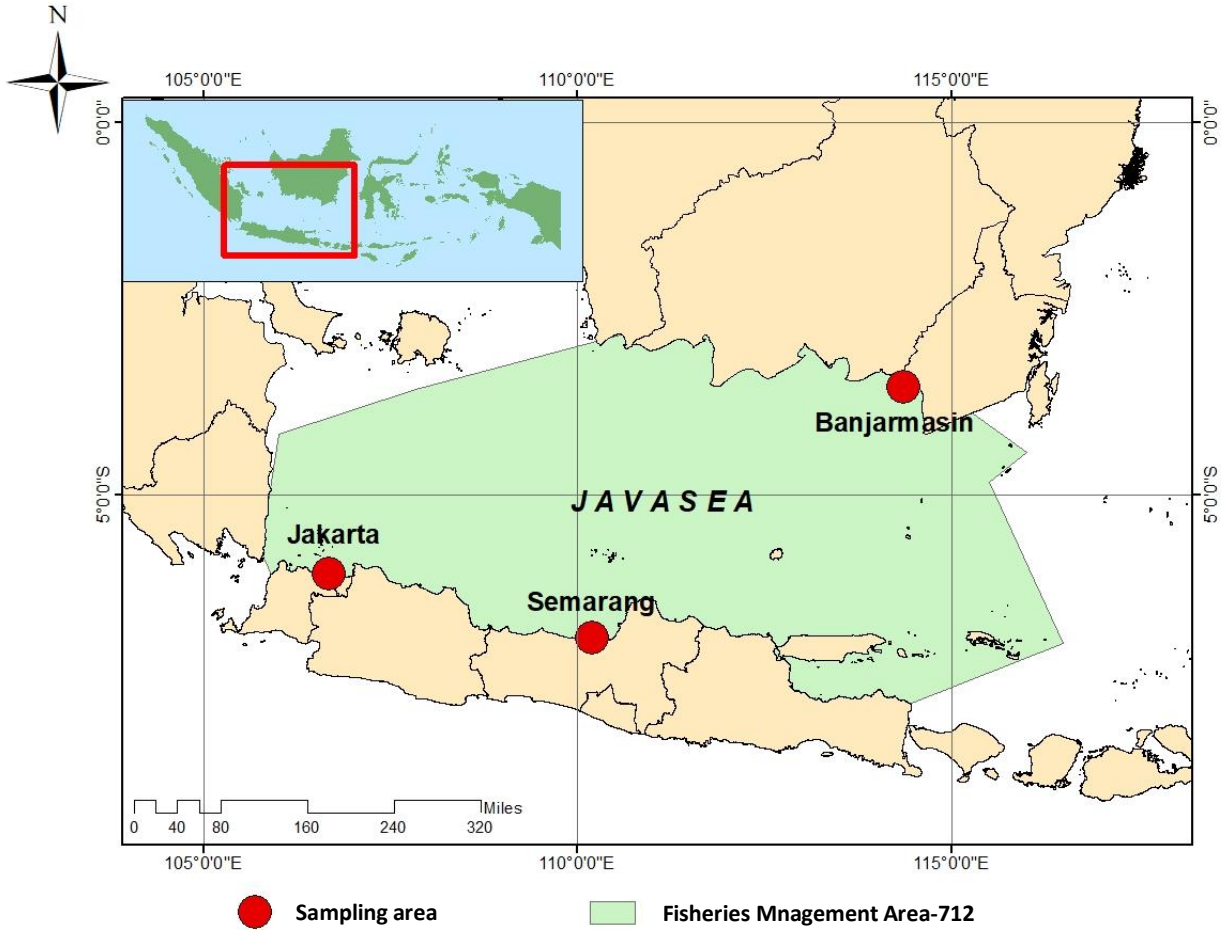

Figure 1. Sampling location of Longtail Tuna (*Thunnus tonggol*) in Java Sea, Indonesia

PCR reaction was carried out in the volume of 25 µL, using 3 µL DNA template. PCR reaction and thermocycling profile modified from method in Allen et al. (2017): an initial denaturation of 94 ℃ for 15 s, 38 cycles of 94 °C for 30 s, 50 °C for 30 s, 72 °C for 45 s, with final extension of 72 °C for 5 min. PCR reaction were included in 25 µL reaction volume, with 2.5 µL 10X PCR Buffer (Applied Biosystems), 2,5 µL 10 mM dNTPs, 1.25 µL of each primer at 10 mM, 2 µL 25 mM $MgCl_2$ solution, 0.125 µL AmplyTaq Red™ (Applied Biosystems), 13 µL ddH_2O, and 3 µL of DNA (chelex 10%). PCR product visualized using 1% of agarose gel stained by Biotium® *gel red stain*. The PCR product that successfully amplified (Table 1) was sent to DNA Sequencing facility and sequenced using Sanger sequencing methods.

Sequencing editing and alignment was conducted using MEGA6 software (Tamura et al. 2013). The phylogenetic tree was constructed using Bayesian method with MrBayes 3.1 program (Huelsenbeck and Ronguist 2001). The best fit model of molecular evolution for each portioning data was determined using Bayesian Information Criterion (BIC) in jModeltest program (Posada 2008). HKY+I+G was selected as the best model. The Markov Chain Monte Carlo (MCMC) analysis with a random starting tree was run for 10 million generations and sampled every 1000 generations. Comparison with the Genbank database (www.ncbi.nlm.nih.gov) using BLAST (Basic Local Alignment Search Tools) was also conducted to accurately identify the sample as *T. tonggol* species. In BLAST result, identity cover value of 99-100% and query cover value of 98-100% was used as the cut-off value for the similar species.

Genetic diversity, haplotype distribution, and genetic differentiation analysis from between locations were analyzed using 1000 permutation in each significance tests (p-value<0.05) such as Ka*,Kst*, Z*, Snn, and Fst, which were analyzed using DNASp 6 (Rozas et al. 2017). Meanwhile, population genetic structure (Φ_{ST}) between locations (Semarang, Banjarmasin, and Jakarta) were analyzed using Analysis of Molecular Variance (AMOVA) with 10000 replicates permutation in Arlequin Ver.3.5 (Excoffier and Lischer 2010).AMOVA analysis used the significance level of 5% (p-value < 0.05). In this analysis, Φ_{ST} is used as an analog of Fst that combines genetic distances between sequence data to know the allelic relationship among locations (Excoffier et al. 1992).

RESULTS AND DISCUSSION

Among all the samples collected, there were differences in the number of samples in each region due to the difficulty in samples collection, which caused by the fishing seasons, the type of artisanal fishing boat used to collect the fish, and also the limitation of areas the Longtail Tuna (*Thunnus tonggol*) found. Longtail Tuna is known as neritic species of tuna and can be found in the shallow water near coastal areas, therefore this type of tuna species can be found in the area of Java Sea. However, the similar morphological characters this species has with other tuna, has also increased the difficulties in identifying the fish as the correct species, which thus lead to mislabeling it with other tuna species (Pauly and Froese 2012).

Molecular genetic has been known to be a powerful method to help identify species with cryptic morphological characters (Valentini et al. 2009; Bucklin et al. 2011). In this study, we used mitochondrial (mtDNA) control region marker to help identify the correct species of Longtail Tuna (*T. tonggol*), and used a similar marker to study its population structure. This marker was chosen because of its high polymorphic rate and has been widely used to identify species of tuna and Scombridae family (Menezes et al. 2012; Kumar et al. 2016).

In this study, Chelex method was used in DNA extraction method, because this method has been widely known as a simple, non-hazardous, and rapid genome DNA extraction process compared to other methods (Griese and Linder 1994). Chelex methods have been successfully used to extract good quality of DNA and commonly used methods of DNA extraction for genetic study, especially for commercial tuna such as *Thunnus obesus* (Pertiwi et al. 2017), *Thunnus albacares* (Akbar et al. 2014), *Auxis thazard*, *Euthynnus affinis*, *Katsuwonus pelamis*, *Rastrelliger kanagurta*, and *Scomberomorus commerson* (Jackson et al. 2014). All of the sequences generated from the samples were deposited in the Genbank database (http://www.ncbi.nlm.nih.gov) with accession no. MT542205-MT542319.

Phylogenetic tree

Phylogenetic tree result from Bayesian method showed that all the samples from three populations (Jakarta, Semarang, and Banjarmasin) were mixing into one clade (Figure 2). A sequence of *Thunnus albacares* (yellowfin tuna) (accession no. JN572792.1), and *Euthynnus affinis* (accession no. JN5655157.1) were used as an outgroup to distinguish the Longtail Tuna samples with other species (Wang et al. 2012).

This study showed a similar pattern as the bigeye tuna (*Thunnus obesus*) population in Indonesia which indicated one major clade of bigeye population and minor genetic differences between specific locations (Pertiwi et al. 2017). Meanwhile, research conducted by Willette et al. (2016) on Longtail Tuna in several locations in South China Sea, i.e. Indonesia, Vietnam, and Philippines indicated that there was no specific pattern of population difference between locations and population, therefore was considered as a mixing population. Although several tuna species showed a mixing population, there was also an indication that some species showed the signal of genetically differentiated populations, such as the Skipjack study (*Katsuwonus pelamis*) done by Menezes et al (2012). In this study for the skipjack population of Indian coast showed that there are four different clades of the Skipjack Tuna within the Indian waters.

Genetic diversity and genetic structure

Genetic diversity result showed haplotype diversity value (Hd) of 0.99268 and nucleotide diversity (π) value of

0.02217. This value of genetic diversity were both considered as high value, compared with the result conducted by Willete et al. (2016) and Kunal et al. (2014). Genetic diversity value of each of the populations is shown in Table 1.

The haplotype distribution also indicated that there was haplotype sharing between population of *T. tonggol* in Java Sea (Figure 3), with 9 haplotypes shared only between two populations (Jakarta-Banjarmasin, Jakarta-Semarang, Semarang-Banjarmasin), and 1 haplotype was shared among all three populations. The genetic differentiation result indicated that there are no significant differences between locations (p-value < 0.05) (Table 2). The lowest value of Fst was found between Banjarmasin and Semarang (Fst: 0.0021) compared to other locations. Population genetic analysis using Analysis of Molecular Variance (AMOVA) showed the value of Φ_{ST} is 0.00328 (*p-value*>0.05) (Table 3), which indicated non-significant population structure.

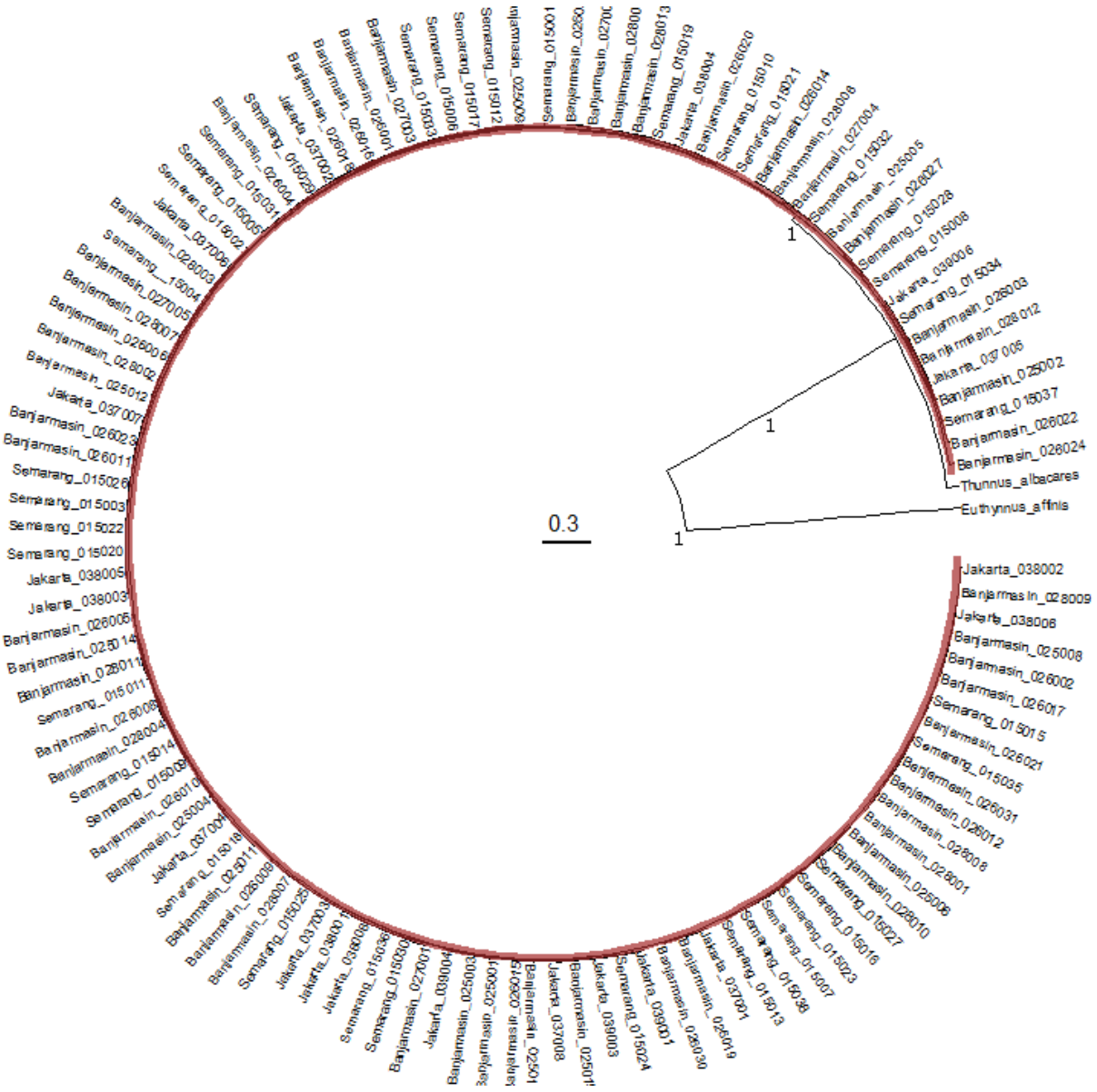

Figure 2. Phylogenetic tree of *Thunnus tonggol* samples from three locations, analyzed using Bayesian methods. The node number represent posterior probabilities value. *Thunnus albacares* (accession no. JN572792.1) and *Euthynnus affinis* (accession no. JN5-655157.1) were used as an outgroup.

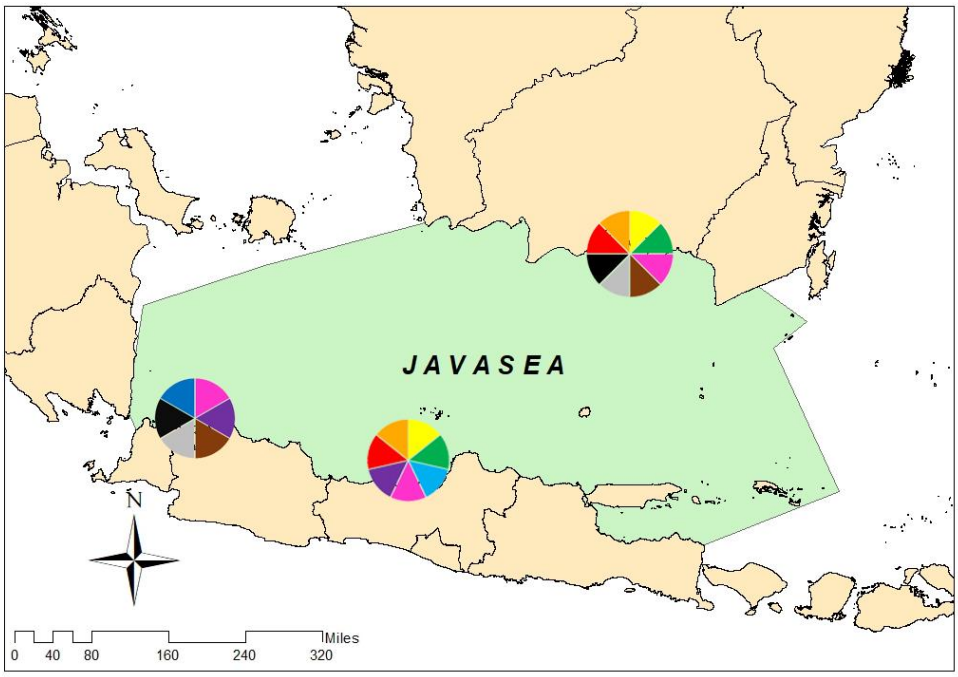

Figure 3. Haplotype distribution of Longtail Tuna (*Thunnus tonggol*) in Java Sea, Indonesia

Table 2. Genetic differentiation estimates for geographical locations of Longtail Tuna (*Thunnus tonggol*) in Java Sea, Indonesia

Location	Ks*	Kst*	Ks*.Kst* P-value	Z*	P-value	Snn	P-value	Fst
Banjarmasin/Jakarta	8.3865	0.0004	0.262	6.9882	0.373	0.6732	0.167	0.0025
Banjarmasin/Semarang	6.4474	0.0010	0.311	7.4256	0.158	0.5958	0.078	0.0021
Jakarta/Semarang	5.8367	0.0039	0.162	6.3728	0.163	0.6083	0.162	0.0056

Table 3. Analysis of Molecular Variance (AMOVA) of *Thunnus tonggol* in Java Sea, Indonesia

Source of variation	DF	Sum of squares	Variance component	Percentage of variation
Among population	2	17.359	0.03204 Va	0.33
Within-population	122	1097.815	9.80192 Vb	100.33
Total	114	1115.174	9.76987	
ΦST	0.00328			
P-value	0.56554 ± 0.0070			

This result indicated that *T. tonggol* populations in Java Sea were considered as a single group (based on phylogenetic and Φ_{ST} result), with high genetic diversity value showed in its high haplotype diversity and nucleotide diversity. High haplotype diversity also indicated by the high number of unique haplotypes between each of population. The result in our study showed similar high haplotype diversity with the study conducted by Kunal et al. (2014) in India that indicated a high genetic diversity value of 0,99. A similar study using different species of tunas, i.e. Bigeye Tuna (*Thunnus obesus*) by Pertiwi et al. (2017) in Indonesia, Yellowfin Tuna (*Thunnus albacares*) by Akbar et al. (2014) in Mollucas sea, and Skipjack Tuna (*Katsuwonus pelamis*) by Manezes et al (2012) in Indian Coast, also showed that several species of tuna that has an indication of high genetic diversity.

Population genetic analysis using AMOVA showed that *T. tonggol* populations in Java Sea were not significantly different and consider as a single population, which showed similar pattern with the result of *T. tonggol* conducted by Kunal et al. (2014) in Coast of India and Willette et al (2016) in South China Sea, although the analysis comparison of Indian and South China Sea population did indicate a distinct clade between those two groups. This resulting study indicated that genetic sharing (gene flow) between population are still happening. The

migration behavior of tuna may become one of the factors that caused this result (Pecoraro et al. 2015), such as *T. tonggol* that known as an ontogenetic migration species (Griffiths et al. 2007), but not highly migratory (Serdy 2004). The oceanographic condition, such as current flow could also be related to strong gene flow between populations (Saleky et al. 2016). However, geographical distance would not give real effect to the genetic flow on migratory species such as tuna (Akbar and Aris 2018). Furthermore, tuna larval stage can also travel at a great distance of <50 km (Green et al. 2014). Although *T. tonggol* is known as neritic tuna and commonly found in shallow water, the characteristic of Java Sea with the maximum depth of 70 m (Nurhakim et al. 2007) are suitable habitat for *T. tonggol*

This study can be used as a preliminary result for population stock of *T. tonggol* in Java Sea using genetic approach. Based on the resulting study, integration of policy and fisheries regulation for *T. tonggol* in Java Sea are needed, especially between each fisheries landing site within Java Sea and FMA-712 (WPP-712), due to the high genetic connectivity between populations of *T. tonggol* in Java Sea. Despite the high genetic diversity of *T. tonggol* in Java Sea, a serious threat in one of its populations can have an impact on the other populations. In the study done by Griffiths et al. (2010), it is showed that *T. tonggol* has more slow growth rate and live longer than other *Thunnus* that have the similar size, such as Blackfin Tuna (*Thunnus atlanticus*), dan Bigeye Tuna (*Thunnus obesus*). This fact has been indicated that the Longtail Tuna should be protected for its population sustainability.

In one of the *T. tonggol* study in Java Sea, it is mentioned that the conditions of this species in the areas are listed as fully exploited (Restianingsih and Hidayat 2018), which then rise a sustainable fisheries issue because Indonesia and other countries such as Thailand, Malaysia, and Iran are the countries that have a high contribution on the *T. tonggol* fisheries in the world (Griffiths et al. 2010; Abdussamad et al. 2012; Darvishi et al. 2018). Another study conducted in Southwest Aceh indicated that the condition of *T. tonggol* population in that area indicated a low vulnerability status and high productivity rate, which signifies that the Longtail Tuna population in Southwest Aceh is still in a good level of resource sustainability category (Rahma et al. 2019). Therefore, further research regarding the vulnerability status of *T. tonggol* population in Indonesia is needed to assess the sustainability of this species.

In conclusion, Longtail Tuna (*Thunnus tonggol*) population within the three different locations in Java Sea (Jakarta, Semarang, and Banjarmasin) showed non-significant differences and indicated as a single mixed population. The study would help fisheries managers and conservation managers to uphold management based on location, especially in Java Sea. However, further study is needed to understand the connectivity of Longtail Tuna between all of Indonesia's Fisheries Management Areas (FMA) to protect the sustainability of Longtail Tuna population.

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