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Genetic Population Structure of Yellowfin Tuna (*Thunnus albacares*) as Based Data of Fish Conservation in North Mallucas Sea

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ABSTRACT

Tuna is a migratory species with high economic value. Utilization of tuna is increasing and growing every year. Fishing intensity of tuna in the Maluku Sea is high and can disrupt the population. The existence of the population will be threatened if not managed properly. The latest genetic information approach is necessary to prevent the population decline. The purpose of this research was to know the genetic structure of yellowfin tuna in North Maluku waters, Indonesi. Sampling was conducted in May-July 2016 in Morotai Island, Obi and Sanana, while secondary data was gathered in Ternate Island, Bacan and Ambon. A total of 72 samples were collected and analyzed. The result of the study found that the base length (bp) of the control region of mtDNA was found to be 512 bp (base pairs). the genetic distance in the nearest population is at Sanana and Obi (0.025). The results of genetic distance analysis between population found genetic similarity between Morotai-Sanana (0,021), Obi-Sanana (0,025), Obi-Morotai (0,026) and Ambon-Sanana (0,026), while the furthest genetic distance was found in Ternate-Bacan (0,040) and Ternate-Obi (0,042). The pairwise comparison test (Fst) shows a few genetic differentiation between yellowfin tuna populations. The value (Fst) of the yellowfin tuna population shows a strong gene flow between populations. The haplotype distribution shows a relationship between haplotypes in both yellowfin tuna, thus failing to show clade between different geographic locations. Unsustainable use can harm the population through genetic quality. Several approaches should be taken to support the life cycle of yellowfin tuna. The overall result shows that there has not been any change of genetic structure of yellowfin tuna in North Maluku Sea.

Keywords : *Haplotype, genetic distance, North Maluku, yellowfin tuna, pairwise comparison test*

1. Introduction

Tuna fish resources have important economic value, widely spread almost throughout the Indonesia waters (Sibagariang et al, 2011). Tuna is an important commercial species that represents a total of 8% of world fish export with a total of 6.6 million tonnes (FAO, 2012; Nikolic and Bourjea, 2012). The total fishing production of tuna in Indonesia is 15.26 million tons (Seafish, 2015). The largest contribution to tuna industry in Indonesia is Pacific Ocean 80% and Indian Ocean 20% (FAO, 2010). This high tuna potential is due to Indonesian waters being between the Pacific and Indian Oceans (Koesmawati et al, 2015). Species of tuna fish caught in Indonesian waters include skipjack, large eye tuna, yellowfin tuna, tuna alalunga (KKP, 2015; Lailossa, 2015).

Yellowfin tuna is a large pelagic fish that is globally distributed in the equatorial region and migrates into the waters of Indonesia (Nishida et al., 1998; Bailey et al., 2012; Grewe et al., 2015). Within the last few decades, yellowfin tuna has become the target species of fishing operations. The ISSF report (2015) showed the a total catch of yellowfin tuna achieved 14,000.00 tons and was the second highest catching species after skipjack. Sibert et al (2009) reported that yellowfin tuna was the target species of fishing operations in temperate and tropical climates regions.

Indonesian waters, particularly North Maluku waters, have potential resources and as tuna fishing ground (KKP, 2015). Fishing production from North Maluku waters by 2014 was 218,000 tonnes (KKP, 2015). Geographical positioning gives benefits to this waters due to its direct border with the Pacific Ocean, Seram Sea, Maluku Sea, Halmahera Sea and Banda Sea which is the entry point of the current Cross Indonesia (Akbar et al., 2014b). The intensity of tuna fishing certainly gives the fear of over fishing. This kind of activity has an effect on the life cycle, causing a decrease in population (Kawimbang et al, 2012; Akbar et al, 2014b). If there is an increase in fishing intensity then the influence of fishing pressure tends to increase (Sriati, 2011). Seeing the pressure of fishing is quite high, fisheries management becomes a very important tool for maintaining the sustainability of fish resources (Melmambessy, 2010). In addition the most common problem in fisheries is resource management (Montes et al, 2012)

The strategy of yellowfin tuna protection from scarcity, population decline to extinction in the future can be conducted through genetic conservation activities. But the activity begins with the structure of the genetic population. For conservation purposes, marine organisms must be managed at the population level to underpin population persistence and sustainability (Carvalho et al., 2010). Species with an over exploitation category require urgent action to improve management and conservation efforts (Collette et al, 2011a). Knowledge of population structure is important for fisheries management effectiveness, determination of fish conservation, stock improvement and resource conservation (Nishida et al., 1998; Chiang et al., 2006; 2008; Carpenter et al., 2011; Akbar et al., 2014b; Aguila et al 2015). Structural knowledge can be used as a conservation and fisheries management effort for commercial commercially valuable species in Indonesia (Jackson et al, 2014). Efforts to manage fishery resources should be able to maximize profits while maintaining resource sustainability (Sriati, 2011).

Research on the structure of yellow fin tuna genetic population (Thunnus albacares) was reported by Scoles and Graves (1993), Permana et al (2007), Chiang et al (2006), Chiang et al (2008), Nugraha (2009), Moria et al (2009), Wu et al. (2010), Wijana et al. (2010), Kunal et al (2013), Kunal et al (2014), Suman et al. (2013). Analysis of population structure of tuna fish using DNA sequencing technique (Sanger et al, 1977). DNA sequencing is a method for identifying base pairs between different individuals and makes it possible to conclude evolutionary relations (Freeland, 2005). Research on the diversity and genetic structure of yellowfin tuna at North Maluku itself has been done by Akbar et al (2014b; 2015) in the Maluku Sea, Indonesia. However, this research does not yet cover the complete North Maluku waters, so it is require an additional information about the genetic structure in some waters that is useful to see the overall current genetic condition.

2. Materials and Methods

2.1. Samples colections

Collection of tuna samples were done in May-June 2016 in three locations namely Morotai Island (10 samples), Obi Island (10 samples) and Sanana Island (10 samples), North Maluku Province. The samples taken werethe catches from the waters around the research sites landed at Fish Landing Base (PPI) and Fishery Port of Nusantara (VAT) (Figure 1). Secondary data were tuna fish sequences located in Ternate and Bacan, while Ambon was used as a comparison. The overall secondary data was the result of previous research. Samples were then photographed, measured in length and cut the pectoral fin section as long as 3 cm, then stored in a tube containing 96% ethanol for preservation.

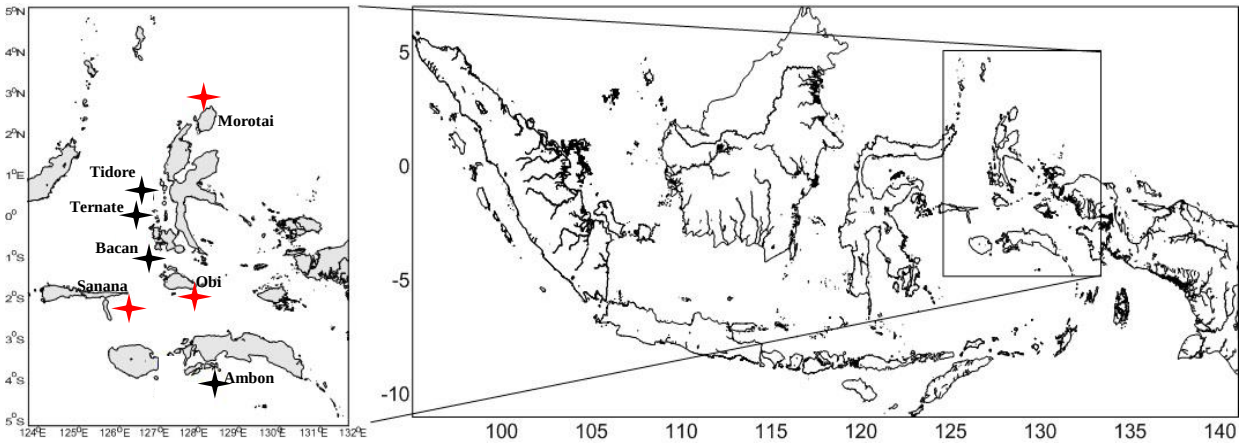

Figure 1. Location of yellowfin tuna sampling (*Thunnus albacares*) in North Maluku Sea, Indonesia (red star = Primary Data, Black star = Secondary Data)

2.2. DNA Analysis

Extraction, polymerase chain reaction, electrophoresis and DNA sequencing DNA samples were isolated with 10% Chelex solution (Walsh et al, 1991). The extraction begins with the inclusion of the sample into the tube then divortex and centrifuged for + 20 seconds. After it is heated using a heat block with a temperature of 95oC for + 45 minutes. Next, tube back divortex and centrifuge for 20 seconds.

The Polymerase Chain Reaction amplification process focused on the locus mtDNA control region with CRK 5'-AGCTC forward primer AGCGC CAGAG CGCCG GTCTT GTAAA-3 'and reverse primers CRE 5'-CCTGA AGTAG GAACC AGATG-3' (Lee et al., 1995). The PCR stages begin with pre denaturation at 94 ° C for 15 seconds, 38 cycles including denaturation at 94 ° C for 30 seconds, annealing at 50 ° C for 30 seconds and extension at 72 ° C for 45 seconds and extension for 72 ° C for 5 minutes. The quality of DNA products PCR results seen with electroporesis. At this stage, a 1 g agarose agar is prepared and added to the erlenmeyer, added 75 mL TAE 1x and heated in the microwave and then added 4 uL EtBr. Afterwards the agarose gel is poured into a mold that has been installed a well-producing comb and is allowed to stand for 30 minutes. The resulting PCR product is sent to the Berkeley Sequencing Facility by the Sanger method (Sanger et al, 1977).

2.3. Data Analysis

Identification of species through the Blast (Basic Local Alignment Tools) application, sequence alignment in order to see the real similarity between sequences with the DNA Weight Matrix ClustalW (1.6) and Translation Weight (0.5) methods were performed with MEGA5 software. Analysis of the structure of the genetic population using Arlequin 3.5 (Excoffier and Lischer, 2009) software with the description of the distance between populations is done using a fixation index (Fst) (Excoffier et al, 1992). Genetic distances within and between populations were analyzed based on distance parameters (Nei, 1972) and (Nei, 1978) and were performed using MEGA5 (Tamura et al, 2011). Distribution of haplotypes and trees between sequences using the Network 4.6 application.

3. Result and Discussion

3.1. Molecular characteristics

The base length bp of the control region of mtDNA was found to be 512 bp (base pairs) using a total of 72 samples, of which 42 samples were secondary data derived from Ternate (12 Samples), Bacan (23 Samples) and Ambon (8 Samples). Similar reports were obtained by Akbar et al (2014b; 2015) ie 517 bp and Kunal et al (2014) at 500 bp. Various studies of tuna species performed showed variations in DNA

fragment size such as Chow and Khisino (1995) of 400 bp, Scoles and Graves (1993) along 304 bp, Chiang et al (2008) with 860 bp, Wu et al (2010) with length 366 bp, Jackson et al (2014) is 400 bp and Fakhri et al (2015) is 532 bp. Some marine organisms also obtained a fairly good length of DNA fragments, as Barber et al (2006) found 625 bp in stomatopods, Allen et al (2013) found 792 bp in walking sharks (Hemiscyllium halmahera), Prehadi et al 2014) and Sembiring et al (2015) obtained 600-700 bp from shark samples, Guan et al (2014) 582 bp for Epinephelus septemfasciatus groupers, Jefri et al (2015) found 526 bp of Epinephelus spp grouper species, Kusuma et al (2016), ie 750 bp on soft corals Sarcophyton trocheliophorum and Saleky et al (2016) obtained 656 bp on turbine type gastropods. The primary length difference was due to the use of different samples and DNA quality, but did not show any effect on sequence analysis results in each sample (Akbar et al, 2014b; Jefri et al, 2015).

3.2. Genetic population structure

Analysis of population structures involving genetic distances and Fixation Index analysis (Fst) and haplotype distribution showed an association between populations. Statistical tests are described as a whole (Tables 1 and 2). Analysis shows that the genetic distance in the nearest population is at Sanana and Obi (0.025). The results of genetic distance analysis between population found genetic similarity between Morotai-Sanana (0,021), Obi-Sanana (0,025), Obi-Morotai (0,026) and Ambon-Sanana (0,026), while the furthest genetic distance was found in Ternate-Bacan (0,040) and Ternate-Obi (0,042). However, when described as a whole, the results obtained show that there was no real genetic distance between all populations. So it could be concluded that genetics between all populations were interconnected among others. In addition, the overall results obtained indicated that all populations were closely related.

Table 1. Genetic distances in and between yellowfin tuna populations

Genetic distances	**Location**	**Morotai**	**Sanana**	**Obi**	**Bacan**	**Ternate**	**Ambon**
Within population	Morotai	0,029	-	-	-	-	-
	Sanana	-	0,025	-	-	-	-
	Obi	-	-	0,025	-	-	-
	Bacan	-	-	-	0,034	-	-
	Ternate	-	-	-	-	0,048	-
	Ambon	-	-	-	-	-	0,027
Between Populatin	Morotai	-	-	-	-	-	-
	Sanana	0,021	-	-	-	-	-
	Obi	0,026	0,025	-	-	-	-
	Bacan	0,031	0,030	0,035	-	-	-
	Ternate	0,039	0,037	0,042	0,040	-	-
	Ambon	0,028	0,026	0,033	0,030	0,038	-

Table 2. . The paired distance value (Fst) at each location

Location	Morotai	Sanana	Obi	Bacan	Ternate	Ambon
Morotai	-	-	-	-	-	-
Sanana	0.983	-	-	-	-	-
Obi	0.972	0.998	-	-	-	-
Bacan	0.965	0.978	0.997	-	-	-
Ternate	0.996	0.968	0.994	0.998	-	-
Ambon	0.995	0.998	0.998	0.995	0.997	-

The proximity of kinship relations between populations may be due to inter-population that have the same parent origin and genetic proximity relationship (Iskandar et al 2010; Kusuma et al 2016). The genetic distance found was also obtained by Scoles and Graves (1993) in the Pacific Ocean also found no significant genetic differentiation between yellow fin tuna, Dammannagoda (2007) in waters (Sri Lanka and Indian Ocean) and Akbar et al (2015) in the Maluku Sea . Genetic distance for other tuna species was also reported by Martinez (2006) in Guinea, Canary, Azores, Canada, Indian Ocean and Pacific Ocean, Chiang et al, (2008) inter at Cocos island, southeast Indian Ocean, Southwest Indian Ocean, Seychelles , the western Pacific Ocean and the islands of Guinea, Martínez and Zardoya (2005) between the Gulf of Guinea, the Canary Islands, the Azores and Canada located in the Atlantic Ocean, Grewe and Hampton (1998) in Ecuador and the Philippines, Wijana and Mahardika (2010) Phiplipina and Spayol, and Suman et al (2013) in the Indian Ocean in western Sumatra and southern parts of Java and Nusa Tenggara. The report about the proximity of genetic distance from other marine species was by Kusuma et al., (2016) onsoft coral Sarcophyton trocheliophorum in Indonesia, Jefri et al, (2015) with species of Epinephelus spp grouper in Indonesia and Saleky et al (2016) i.e from two species of marine gastropods in Papua, Indonesia. Genetic proximity was strongly influenced by flow patterns, high larval spread, appropriate habitat conditions and migration capabilities (Grant, 1985; Lin and Liu, 2008; White et al., 2010; Akbar et al., 2015; Jefri et al., 2015; Kusuma et al., 2016).

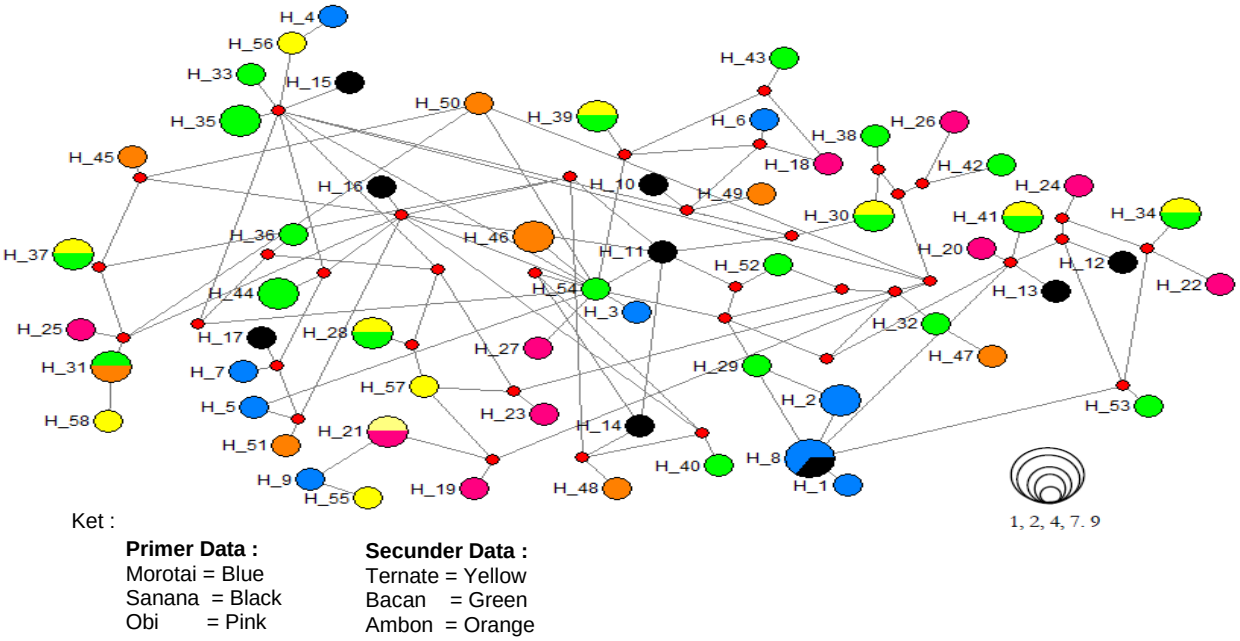

Figure 2. Distribution of haplotipe network of yellowfin tuna (Thunnus albacares) in of North Mallucas Sea

Statistically using pairwise comparison test (Fst) showed a low genetic differentiation between yellowfin tuna populations. The value (Fst) of the yellowfin tuna population showed a strong gene flow between populations. A large gene flow indicated the interplay of populations (Akbar et al, 2015). In addition to genetic and isolation flow was the impact of geographical distance and complex environmental conditions, thereby enhancing strong genetic relationships and no significant differences between populations (Jefri et al., 2015; Kusuma et al., 2016; Saleky et al., 2016). Population size, physical limitations and migration ability might affect the genetic flow (Bremer et al., 2005; Ely et al. 2005; Ward et al. 1994; McQuinn 1997; Nesbo et al. 2000). Several reports of tuna species have also found low genetic deferences both within and between populations in the Pacific and Indian Oceans (Bremer et al 1998, Grewe and Hampton, 1998; Chow et al., 2000; Appleyard et al., 2002; Durand et al., 2005 ; Chiang et al., 2008; Wu et al., 2010; Suman et al., 2013, Jackson et al 2014). Research on other marine species also found the presence of gene flow between populations such as by Jefri et al (2015) with Epinephelus spp grouper, Saleky et al (2016) with sea gastropods and Kusuma et al (2016) on soft coral Sarcophyton trocheliophorum. Although genetic differentiation was very different, there is a genetic flow between populations. The results of the analysis supported tissue distribution and genetic distance in yellowfin tuna populations. Patterns that were formed show a global type of this species met each other and gave a real effect on the genetic flow. Geographical distances did not show any real effect on migratory species movements such as tuna.

The haplotype distribution showed a relationship between haplotypes in both the yellowfin tuna, thus failing to show clade between different geographic locations (Figure 1). The results obtained were supported by analysis of genetic distance and pairwise comparison test (Fst). Haplotype diversity levels were also found in yellowfin species captured at other sites (Chiang et al 2006; Santos et al 2010; Suman et al 2014; Akbar et al 2015). Individual mixing between two populations of species in this region is highly possible due to the high species mobility, prevailing current patterns and larval dispersal distributions (Wyrtki 1961). Ward (1995) explained to show low population differences, migrations involving multiple individuals per generation can be key to generating far-reaching genetic homogeneity. The results indicated that these two fish populations were one offspring and migrated with a migration pattern in the same location causing these two populations to be genetically similar. It also explained that although each population group was separated from one another, these two populations had a genetic proximity and a common ancestor of origin. The geographic position and the presence of barrier and current strength did not limit the distribution of tuna, so it couyld be explained that tuna fish did not have geographical distribution limits.

3.3. Conservation of tuna fish resources

The most widely traded tuna species are yellow fin tuna (T.albacares), large eye tuna (T.obesus), albacore tuna (T.alalunga), Cakalang (K.pelamis), Tongkol (T.tongol). Data and results of Collette et al. (2011b) and Bailey et al (2012) explained that some of the statuses of tuna fish include Fully exploited, Overfihing, Bluefin tuna (Endangered) and Bluefin Tuna pacific (Critically endangered). The high genetic diversity found in the waters of North Maluku might indicate that tuna populations have the ability to cope with environmental changes at any time, as well as to show that the genetic structure of tuna populations remains undisturbed. The higher the genetic diversity, the greater the chance to adapt to the environment (Perwati, 2009). Besides it has a role to improve the productivity of species so that the economic value to support food security (Slamat et al 2011).

Unsustainable use of tuna resource can harm the population through genetic quality. Akbar et al (2014a) says the key to preserve genetic diversity can be done in a preventive way such as firstly, providing regulation on the implementation of minimum catch size limits on every fishing operation, where fish caught are adult fish with a predetermined size proportion. The second is related to the enforcement of time management rules at the time of fishing, where the fishing operation is only done for six months or in peak season. So as to avoid the occurrence of overfishing, tuna fish resources can be guaranteed its sustainability. Third is the creation of a reservation area (reservation region) that is protected by related authorities and involving the surrounding community. This area is important in genetic conservation activities because it plays a role to maintain the survival of tuna populations in waters.

4. Conclusion

The overall results found there are strong proximity and distribution of gene flow among yellow fin tuna populations in the waters of North Maluku, Indonesia. The mixing of haplotypes and distribution illustrates that there is strong genetic mixing among the population. It needs social approach, awareness increase and regulation to strengthen conservation system. In general the population structure is still in good condition.

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