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Genetic Diversity and Population Structure of Bullet Tuna (*Auxis rochei*) from Bali and Its Adjacent Waters

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ABSTRACT

Bullet tuna (*Auxis rochei*) dominates the neritic tuna catch, especially from the purse seine fleet within the western and southern Indonesian waters. However, high catches can lead to stock depletion and lower genetic diversity due to possible inbreeding. Therefore, population genetic information is important in monitoring the sustainability of fish stocks and proposing an appropriate species-specific conservation strategy. This study aimed to analyze the genetic diversity, population structure, and kinship relationship of bullet tuna in Bali and its adjacent waters. Sampling was carried out in September 2020 at landing sites/ports representing the north, east, south, and west region, whereas at least 30 samples were acquired at each location. The result showed that the DNA concentration obtained could produce DNA bands with allele length ranged from 94-260 bp. Observed heterozygosity (Ho) was around 0.440-0.627. While the expected heterozygosity (He) was between 0.932-0.945. The genetic variation among population, within-population, and individuals was 0.36%, 41.04%, and 58.60%, respectively. The results of the analysis of genetic diversity between individuals in the population showed very high genetic diversity. The population structure of the bullet tuna landed in West Bali, East Bali, South Bali and North Bali is the same population stock. The kinship relationship indicates that the four populations are closely related genetically.

1. Introduction

The neritic tuna managed in the TCT RPP consists of four types of neritic tuna including bullet tuna (*Auxis rochei*), frigate (*Auxis thazard*), kawakawa (*Euthynnus affinis*) and longtail tuna (*Thunnus tonggol*) (Suryaman *et al.* 2017). Bullet tuna is the dominant species caught within coastal areas by small-scale or artisanal tuna fishery (Naderi 2016). Neritic tuna are mostly found in the tropical waters of the Indo-Pacific. Even though they live in the ocean, tuna prefers to be near the coast and even juveniles of these fish can be found in bays and harbors (Agus 2017).

According to Sastra *et al.* (2018), the population of neritic tuna is widespread in almost all Indonesian waters, including Bali and its surrounding areas. The Bali Strait itself holds a potential supporting system of aquatic marine life for coastal fisheries communities (Syah *et al.* 2020). Best scientific total catches estimation of bullet tuna from the Indian Ocean in 2019 reaching 24,000 tons with an average (2015-2019) of around 19,000 tons (IOTC-WPNT11 2021). At least 34% (~6,000 tons) was contributed from the Indonesian fleet during the same period (IOTC-WPNT11 2021), in which a small part of the catch were generated from the Bali Strait (Prayoga *et al.* 2017). The high catch of bullet tuna reported in the last five years is shadowed by the uncertainty of catch estimation rather than representing actual condition (IOTC-WPNT11 2021). However, the significant rise of bullet tuna production indicates intensive fishing activities driven by increasing market demand and could potentially cause overfishing of local depletion. Constant monitoring by observing some of the biological parameters of the bullet tuna population is pivotal in keeping its resource in check. One of the tools is population genetics, where one of its main purposes is to investigate genetic diversity. According to Nugraha *et al.* (2016) genetic information can determine the right conservation strategy in a population. In addition to conservation and management of fish stocks, genetic diversity is also a very important factor because the improvement

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of genetic quality is based on the genetic diversity possessed by a population (Sundari *et al.* 2018). It can determine whether there is a genetic transfer between populations to assess the stock status of the population. Zedta and Setyadji (2019), obtained the results of visualization of PCR products with Aro2–38 microsatellite DNA primers on bullet tuna and frigate tuna showed DNA bands in samples that were successfully amplified from all loci, but not all samples showed the same thickness of DNA bands. This marker can be a useful tool for use in population genetic studies of bullet tuna species and other fish of the same genus.

Therefore, due to the lack of information regarding its genetic variability and population structure, especially in Indonesian waters, this preliminary study intended to examine the genetic diversity, population structure, and kinship of bullet tuna. Especially those landed in landing sites/fishing ports scattered around the island of Bali. Such information is essential for conducting a better harvest strategy in the future.

2. Materials and Methods

2.1. Sample Collection

Tissue sampling was carried out in September and October 2020 on four locations representing the waters around Bali, namely PPN Pengambengan on the west, TPI Karangasem on the east, TPI Bondalem Buleleng on the north, and PPI Kedonganan on the south (Figure 1). The length of the journey from land to the fishing area ranges from 1-2 hours so it can be ascertained that it is still in the waters of Bali and its surroundings. Samples were taken in the form of slices of meat from the pectoral to dorsal parts of bullet tuna fish as many as 30 samples per location. The tissue was taken using a cutter and then put into a vial filled with 96% alcohol.

2.2. DNA Extraction

DNA extraction was carried out using the DNeasy Blood and Tissue Mini Kit. The extraction process is according to the instructions from the DNeasy

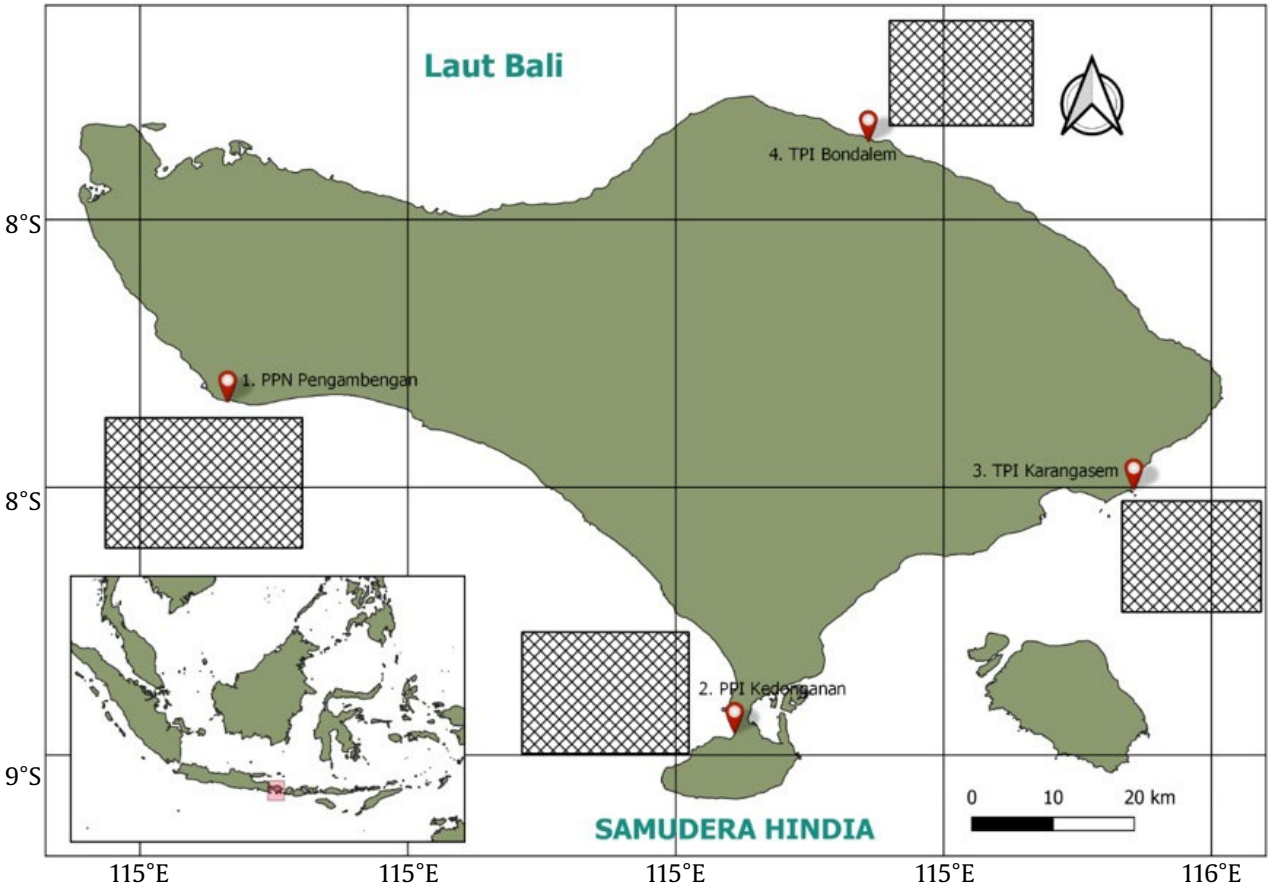

Figure 1. Sampling location of bullet tuna (*Auxis rochei*) in around Bali and its adjacent waters

Blood and Tissue Mini Kit. The extracted DNA was then measured for its concentration using a nanophotometer. If the amount of concentration in the DNA tissue that has been extracted is low, the extraction process will be repeated again.

2.3. DNA Amplification

Amplification in the nucleus of DNA cells was carried out using five microsatellite DNA primers (Catanese *et al.* 2007), as shown in Table 1. The PCR amplification process used a combination of Red Mix, NFW (Nuclease-Free Water), primer F, primer R, and DNA template with a total concentration of 25 µl. The amplification temperature configuration used is as follows: pre-denaturation at 95°C for 2 minutes for one cycle, followed by 34 cycles of denaturation at 95°C for 30 seconds, annealing with temperature and time based on Catanese *et al.* (2007), extension at 72°C for 45 seconds and one final extension cycle at 72°C for 5 minutes.

2.4. Electrophoresis Using QIAxcel

The microsatellite locus polymorphism screening process was conducted using the QIAxcel fragment analysis tool. It uses a high-resolution DNA screening gel cartridge with a size marker ranging 25-500 bp, with an alignment marker measuring 15 bp/600 bp (Qiagen 2017). Band pattern data and electrophotogram were analyzed using QIAxcel Biocalculator software to score the alleles that emerged. The result was used to measure several population genetic parameters, including the number of alleles, allele frequency, heterozygosity, variability (Ho/He), genetic distance, kinship, and population structure.

2.5. Data Analysis

Allele variety, population structure, and genetic diversity were calculated using the Arlequin version 3.5 program (Excoffier *et al.* 2005). Relationships between populations were determined based on genetic distance parameters calculated according to Slatkin M. (1995). Differences in genetic diversity among the population were estimated using Analysis of Molecular Variance (AMOVA) to determine genetic variation and population structure between population groups of bullet tuna (*A. rochei*). Meanwhile, GenAlEx software version 6.5 (Peakall and Smouse 2012) was utilized to determine the polymorphic locus.

3. Results

3.1. Genetic Diversity

Measurement of genetic diversity was carried out on 600 PCR products resulting from the extraction of 120 samples of bullet tuna obtained from fishing ports located in West Bali (population 1), East Bali (population 2), South Bali (population 3), and North Bali (population 4). The results showed that the genetic diversity between populations was very high according to the number of alleles for each locus (Table 2). Locus Aro3-37 had the highest average number of alleles (24). In contrast, Aro2-15 had the least mean number of alleles with 16. On the other hand, Aro1-10 produced the longest allele size of 260 bp, whereas locus with the shortest allele size was Aro3-37 with 94 bp. The highest allele frequency among five loci was aro1-10, followed by aro1-59, aro2-15, aro4-13, and aro3-37, respectively (Figure 2).

Table 1. Microsatellite DNA primers for bullet tuna

Locus	Repeat motif	Primer sequences (5'-3')
Aro1–10	$(CA)_4(CTCA)_3(CA)_{23}$	F: HEX-CCCACCCACCCAGCCCTTCC
		R: TCATCCCTTGTTACCTGCGTTTCTATTTTC
Aro1–59	$(CA)_{12}$	F: FAM-CTACGTGCATGTCAGGTTGGATTCA
		R: TTGTCTAAGTTTTTCTCCTGTGCTTTTATTGGTC
Aro2–15	$(CA)_{12}(C)(CA)_8(CTA)(CA)_4$	F: HEX-CCATTTTTTCCTCAAACCAAACTGCCATT
		R: GTGGGTGTGTTGTAAACTCTGAGCAGGTGT
Aro3–37	$(TTCTC)_{20}$	F: CTTTATATTGGCAAGAGTATTGTTCACTCATTT
		R: HEX-TTGAGCCCACATGGTTGATAGCAGGAT
Aro4–13	$(CT)_4(CC)(CT)_{16}$	F: HEX-AATCCATCCATCACACACAGCCAGA
		R: TTAAGTGTATGTGTTTGAGAGACAGAGCGAGA

Table 2. Allele length (bp) and average number of alleles per microsatellite locus

Locus	Number of samples	Allele length (bp)	Number of alleles (average)
Aro1–10	30	174-260	20
Aro1–59	30	112- 170	17
Aro2–15	30	158-206	16
Aro3–37	30	94-190	24
Aro4–13	30	110-190	20

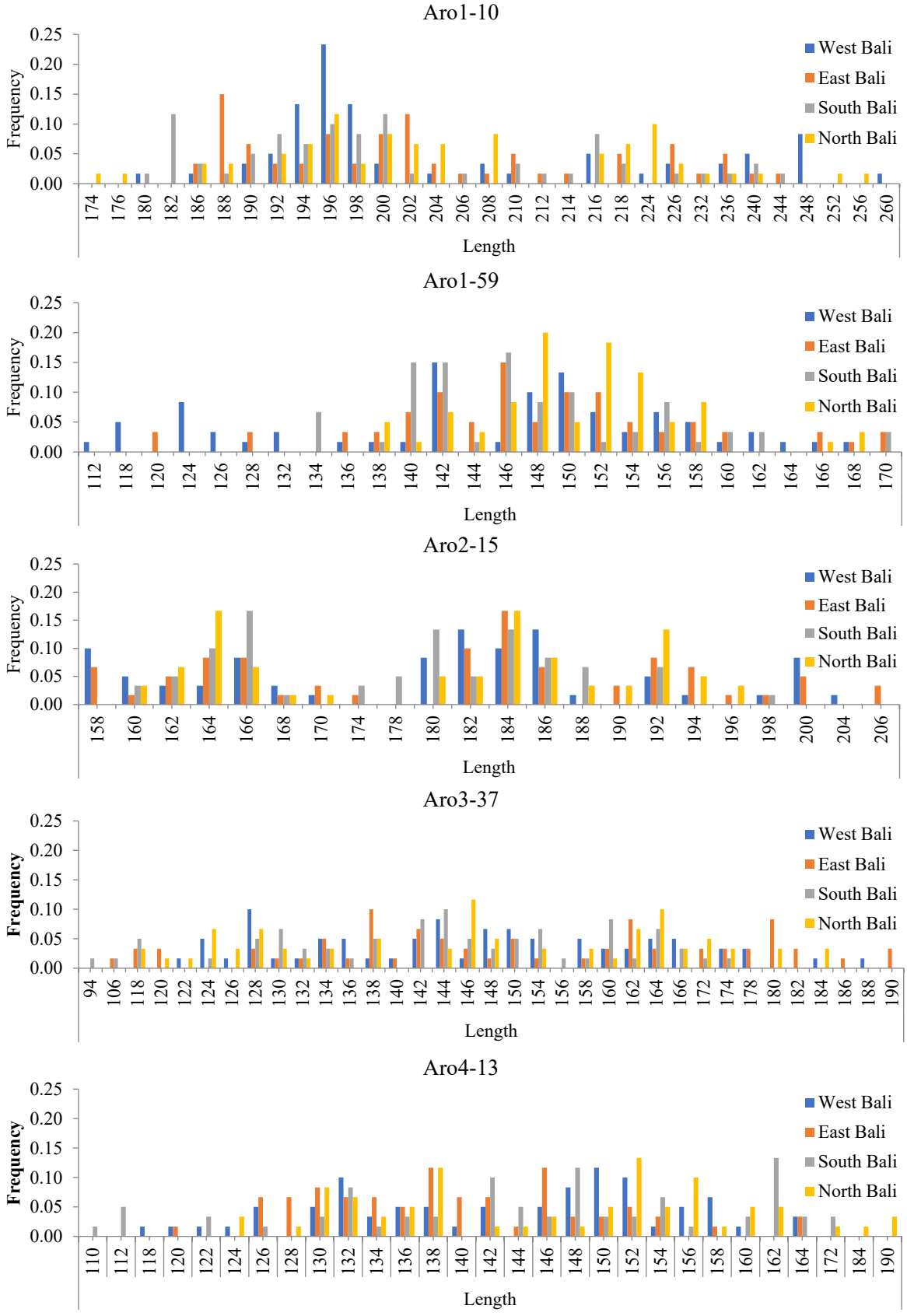

Figure 2. Allele frequencies summary of bullet tuna across all loci and sampling locations

The number of alleles of bullet tuna from the western part of Bali ranged from 17-25, and in the east, it went from 18-27. Meanwhile, between south and north, they had relatively similar values between 13-24. The observed heterozygosity (Ho) and the expected heterozygosity (He) derived from all loci at each location ranged from 0.440-0.627 and between 0.932-0.945, respectively. The highest value for both parameters occurred in the east, and the lowest was in the north (Table 3).

3.2. Population Structure

The fixation index (FST) analysis by using Arlequin showed that there was no genetic differentiation between populations (p-value<0.05) at a 95% confidence interval (Table 4 and 5). This indicates that the bullet tuna populations landed at the four fish landing sites in Bali are still in the same population stock and come from the same parent population and migration pattern.

The average value of the inbreeding rate among the population for bullet tuna (FIS) was 0.41184 (low), while the inbreeding rate within the population (FIT) was 0.41397 (low) and the genetic differentiation (FST) was 0.00362 (very low). Genetic variation among bullet tuna populations from all locations was 0.36%. In contrast, the genetic variation among individuals and within individuals was 41.03% and 58.60%, respectively (Table 6).

3.3. Kinship Analysis

Kinship analysis can be determined based on the genetic distance via DNA band profiles (Table 7). The smaller the genetic distance value obtained, the closer the kinship relationship of the bullet tuna population and vice versa. The bullet tuna landed in the east and northern part of Bali has the closest kinship while between south and north has the farthest. The low genetic distance values indicated that the four populations were closely related.

Table 3. Parameters of genetic diversity of bullet tuna

Locus	West Bali			East Bali			South Bali			North Bali		
	k	Ho	He	k	Ho	He	k	Ho	He	k	Ho	He
Aro 1-10	18	0.333	0.903	21	0.367	0.942	22	0.467	0.943	21	0.267	0.949
Aro 1-59	22	0.700	0.937	18	0.700	0.940	15	0.567	0.908	13	0.433	0.895
Aro 2-15	17	0.433	0.929	18	0.767	0.934	14	0.633	0.916	15	0.533	0.914
Aro 3-37	25	0.767	0.963	27	0.700	0.966	24	0.667	0.959	23	0.567	0.958
Aro 4-13	21	0.600	0.949	18	0.600	0.945	21	0.600	0.946	21	0.400	0.944
Average	20	0.567	0.936	20	0.627	0.945	19	0.587	0.935	18	0.440	0.932

k = Number of alleles, Ho = Observed heterozygosity, He = Expected heterozygosity

Table 4. Matrix of fixation index value (FST) for bullet tuna landed around Bali

Population	West Bali	East Bali	South Bali	North Bali
West Bali	-			
East Bali	0.01079	-		
South Bali	0.01007	0.00960	-	
North Bali	0.01203	0.00661	0.01432	-

Table 5. Matrix of p-value for bullet tuna landed around Bali

Population	West Bali	East Bali	South Bali	North Bali
West Bali	-			
East Bali	0.09473	-		
South Bali	0.15039	0.15430	-	
North Bali	0.14551	0.55859	0.05371	-

Table 6. AMOVA results based on the mean value of loci in the bullet tuna population group

Variation	Sum of Squares	Variant component	Percentage of Variation
Between populations	11.533	0.009	0.362
Between individuals in a population	386.350	0.972	41.035
Between individuals	166.500	1.387	58.603

Table 7. Genetic distance between bullet tuna populations

Population	West Bali	East Bali	South Bali	North Bali
West Bali	-			
East Bali	0.01091	-		
South Bali	0.01017	0.00970	-	
North Bali	0.01217	0.00666	0.01453	-

4. Discussion

Genetic diversity could act as an indicator of a certain condition in the future (Nozawa *et al.* 1982). It could be determined through one of its attributes, namely heterozygosity (Tanabe *et al.* 1999). Based on this study, the genetic diversity of bullet tuna between individuals landed at four fish landing sites in Bali is categorized as high. According to Nei (1987), the values fell between 0.8-1.0. This result was relatively similar to the study from Catanese *et al.* (2007) in the Mediterranean, Atlantic, and Pacific waters. Populations with high genetic diversity have a better chance of survival because each individual responds differently to environmental conditions. The higher the heterozygosity value, the higher the outbreeding, thus increasing the proportion of heterozygous genotypes (Noor 2000). Hendiari *et al.* (2020) explained that the high value of genetic diversity of fish in a population could occur due to two reasons. The first reason is the size of the fish caught and the large number of fish populations in the waters. The second one is related to the high migratory ability of this species.

The mean value of observed heterozygosity (Ho) obtained in this study was smaller than the expected heterozygosity value (He). It indicates the genotypes imbalance in the population (Tambasco *et al.* 2003). Machado *et al.* (2003) added it could be a sign of intensive selection and the possibility of inbreeding mating. Based on those two explanations, it is suggested that all bullet tuna caught in waters around Bali belong to a single population. It has a cosmopolitan-type ability and usually forms a large school. Besides, the spread of bullet tuna also often follows the circulation of sea currents (Agus 2017). Kasim *et al.* (2020) adding that, seasonal migration patterns at the adult fish stage and during the spawning stage will cause the potential for dispersal to be high so that genetic differentiation between populations will be lower. The waters of the Bali Strait are semi-enclosed waters that connect the Bali Sea in the north and the Indian Ocean in the south (Priyono *et al.* 2008). The circulation of water masses in the waters of the Bali Strait enters from the Indian Ocean (south-southeast) towards the Bali Sea (north-northwest) (Pranowo and Realino 2006). Migration activities can also allow cross breeding and mixing of genes between populations (Agus 2017).

Hartl and Clarke (1997), divided the FST value into 4 levels, namely low (<0.05), moderate (0.05-0.15), high (0.15-0.25) and very high (>0.25). Based on these criteria, the bullet tuna in this study were classified as having low genetic differentiation, indicating a strong genetic relationship between the populations. Furthermore, the statement was supported by insignificant differences ($p>0.05$) among four bullet tuna populations around Bali. Unfortunately, the lack of a similar study makes this study's results incomparable. Both inbreeding rate within the population (FIS) and inbreeding rate among the population (FIS) showed they were not significantly different from zero, which implied no sign of migration between the existing population. Moreover, the genetic differentiation value (FST) was close to zero, which illustrates that the genes in each subpopulation still have a fairly high genetic diversity due to the low inbreeding coefficient rate. This means that blood mixing between subpopulations is less likely to occur in closely related mating. Or in other terms, mating still occurs randomly between subpopulations, with no ability to interfere with each other. Further, AMOVA analysis (Table 6) also confirmed that the genetic differences of bullet tuna from all the landing sites were unlikely influenced by the differences between populations but rather caused by differences within and between individuals (41% and 58%, respectively). These results indicate that the genetic diversity between individuals in the population was high, while between populations was low. It indicated genetic mixing between populations, causing similarities in genetic structure. The similarity of gene structures between populations with far geographical distances is caused by several factors, including the similarity of origin (ancestry refugia) (Tsuda *et al.* 2009).

The eastern part of Bali's population has a close relationship with its northern counterpart, whereas the population between the south and north had the most distant relative relationship. Theoretically, bullet tuna migration could be detected in a sequence from the north, toward the east, through the southern part and moved back up through Bali Strait in the west following the Indonesian throughflow

(Arlindo), which flows from the Pacific Ocean to the Indian Ocean (Gordon 2005). In addition, the fishermen's behavior also probably influenced the mixing, where fishing grounds are not fixated in just one area and could be a combination, depending on the seasons. The close kinship of the four bullet tuna populations suggests that these populations come from the same lineage group. High migration mobility results in gene flow due to the greater chance of meeting between populations (Akbar *et al.* 2020). Populations with close kinship have genetic and morphological similarities, possibly due to environmental conditions (Saleky *et al.* 2016).

In conclusion, it is suggested that the bullet tuna population around Bali and its adjacent waters is a single panmictic population. It refers to a random mating technique used by fish in which breeding occurs just as frequently between any two individuals in a group as it does between any two others. Any environmental (e.g., geographic closeness), hereditary (e.g., spawning period), or social interaction does not affect this type of mating (Bahagiawati *et al.* 2006). It was also found in several studies on tuna groups and tuna-like populations (Chiang *et al.* 2008 and Akbar *et al.* 2014).

No distinct population structure was detected for bullet tuna in the western, eastern, northern and southern parts of Bali's waters. Therefore, for future consideration, stock-based assessment of bullet tuna species, especially from Bali waters should be considered as a single stock. The genetic conservation strategy of bullet tuna in Bali based on research that has been carried out can be considered as one large population, so that the exploration of genetic material in the context of *ex situ* and *in situ* conservation can be represented by only one population. Meanwhile, to maintain the stock status of the bullet tuna population in Bali waters, better fisheries management is needed, namely by keeping the bullet tuna migration route the main focus in protecting (conservation) and maintaining the fitness of the bullet tuna fish population. These steps are necessary to ensure the sustainability of fishery resources. Furthermore, the development of Next-Generation Sequencing (NGS) technique for population structure is suggested for higher resolution insight into the population structure of this species.

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