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DNA barcoding and morphometric of *Rastrelliger* spp. in North Maluku Sea, Indonesia

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ABSTRACT

High exploration activity is feared to have an impact to mackerel populations. A sustainable management approach should be taken to provide information about the status of mackerel populations. Study of mackerel population status can be carried out through genetic information. The DNA of the fish samples were collected at traditional fish markets (Morotai, Bacan and Ternate). Laboratory works such as extraction, amplification, electrophoresis and DNA sequencing were analysis at the Indonesian Biodiversity Laboratory (Bionesia). The molecular characteristics of Rastrelliger kanagurta were 374 base pairs (bp). The composition of nucleotides showed the similarity of frequencies between species. Phylogenetic relationship of R. kanagurta in North Maluku Sea suggested that there was any differentiation. The genetic diversity of R. kanagurta was high with a total number of haplotypes and diverse nucleotide diversity. The minimum spawning networks (MSN) found 5 haplotype networks from a total of 12 samples. Morphological measurements of standard length, head height, body width, pectoral fin length and tail were found to have variable values. The length of the weight of the fish is obtained of b = 3, indicating that the growth pattern was isometric or weight gain was equivalent to the growth of the fish length.

Introduction

The waters of North Maluku situate between the two main entrances of Indonesian Throughflow (ITF). The water mass flows carrying a lot of nutrients through the Maluku Sea and Halmahera Sea (Hasanuddin, 1998). Therefore, this water mass flow provides benefits for North Maluku waters. Mackerel (*Rastrelliger spp*) is one of Ternate fisheries commodities with an average total production of more than 438 kg / month which is captured using a mini purse seine and gill net (Gill net) with fishing operation time with the average is 1-2 fishing operations per day (Tangke, 2014). The high activity of mackerel fishing has high relationship with the demand for people's consumption needs every day. Mackerel is a superior commodity that is used by the community for nutritional and protein needs. High exploration activity is feared to have an impact on the population. The process of catching activities carried out continuously will result in population decline and genetic variation in fish (Wigati *et al.,* 2003). Tangke (2014) reported that mackerel caught by fishermen in the waters of Ternate City has a fast growth rate and high mortality rate, so generally mackerel caught is not yet spawned. This illustrates that the use of non-sustainable nature has been carried out, so that it has an impact on fish stocks. A sustainable management approach must be taken to provide information about the status of mackerel populations. Study of mackerel population status can be conducted through genetic information. This analysis aims to determine genetic diversity by looking at whether

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genetic movement occurs between populations so that it can determine the population's living status (Santos *et al.,* 2010). Besides genetic diversity is an important information in the short and long term for a population (Ferguson *et al.,* 1995).

Research on fish genetic in Indonesia has been carried out including genetic studies of large-eyed tuna populations (*Thunnus obesus*) in Benoa Bali (Nugraha, 2009), candra fish (Torsoro) originating from North Sumatra Province and West Java Province (Nugroho *et al.,* 2006), red snapper (*Lutjanus malabaricus*) originating from several fishing areas of the North Coast of Java and the eastern Java Sea (Suwarso, 2002), yellowfin tuna (*Thunnus albacores*) from the regions of Bali, North Maluku and North Sulawesi (Permana *et al.,* 2007), The similar study was also carried out by Wijaya *et al.* (2010) whose fish samples were taken from Spain and Philiphine, betutu fish originating from the Brebes weir reservoir (Susanto *et al.,* 2006), Genetic Diversity in the Population of Beronang Fish (*Siganus guttatus*) in the Makassar Strait and Bone Bay using the random amplified polymorphic reservoir method DNA (Rapd) (Lante *et al.,* 2011), a study of the structure of large-eyed tuna populations in the Indian Ocean, West Sumatra, South Java and Nusa Tenggara, conducted by Suman *et al.* (2013) and Akbar *et al.* (2014) on genetic diversity of yellowfin tuna from two populations in the Maluku sea, Indonesia, Akbar *et al.* (2020) on a preliminary study of the structure of the genetic population of yellow fin tuna from two populations in the Maluku sea, Indonesia, Molecular phylogenetic of grouper collecting from the traditional market (Jefri *et al.,* 2015; Tapilatu *et al.,* 2021; Dwifajri *et al.,* 2022), Sardinella lemuru genetic variant in the strait waters Bali (Kartika *et al.,* 2017) and genetic and phylogenetic variations of yellowfin tuna as a basis for sustainable fisheries management in North Maluku (Aris *et al.,* 2017).

Since the information about mackerel genetic in North Maluku Sea especially around Morotai, Ternate and Bacan sea is not yet available. Even though this fish is a catch that has been available every day since ancient times. This is the reason why this work is essential to be done from genetic aspects. Beside, this study also provide the information on the genetic diversity which can indicate whether there is a genetic transfer between populations or not, so that, it can determine the population's life status of this pelagic species (Santos *et al.,* 2010). Knowledge of genetic population structure is truly necessary in terms of the sustainability and effectiveness of resource management (Chiang *et al.,* 2006; Chiang *et al.,* 2008).

Materials and Methods

Sample collection

DNA samples were collected at traditional fish markets (Morotai, Bacan and Ternate). The number of samples found consisted of Morotai (n = 3), Bacan (n = 3) and Ternate (3 samples) (Figure 1). The samples were then taken the pictures, the 3 cm pectoral fins and so on putting in a tube containing 96% ethanol. Extraction, amplification, electrophoresis and sequencing were carried out in the Bionesia, Bali laboratory. While morphometric measurements of fish include standard length, head height, body width, pectoral fin length and tail.

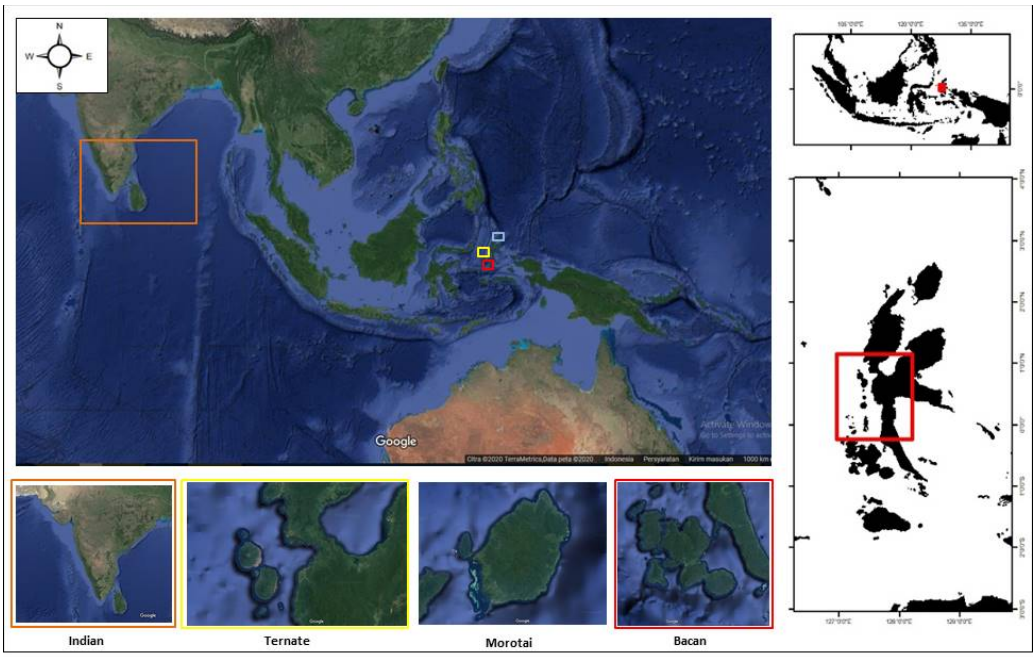

Figure 1. Research Sites *R. kanagurta*. Primer data (Ternate, Morotai and Bacan) and secondary data (Indian).

Extraction, amplification and electrophoresis DNA

Sample extraction was carried out using a Chelex 10% solution (Walsh *et al.,* 1991). Tissue samples were taken of 2 mm using tweezers and inserted into a tube containing chelex solution. Before and after the tissue was removed, tweezers were dipped in 95% ethanol and burned with Bunsen flame. Chelex solution in which sample tissue has been inserted, were vortexed and centrifuged for 20 seconds, then heated in a heating block at a temperature of 95°C for 45 minutes. After heating, the tube is returned to the vortex and centrifuge for 20 seconds. Extraction solution was ready to be used for amplification.

Sequencing

Samples that have been amplified by the PCR method were then sequenced at the sequencing service facility to obtain their nucleotide sequences, using the sanger sequencing method (Sanger *et al.,* 1977).

Data analysis

Data analysis at the Indonesian Biodiversity laboratory (BIONESIA), Bali where the mtDNA region control sequence was analyzed using MEGA5 (Molecular Evolutionary Genetic Analysis) applications (Tamura *et al.,* 2011), Arlequin 3.5 (Excoffier and Lischer, 2009), DnaSP 4.0 (Rozas *et al.,* 2003) and Network 4.6. Secondary data was downloaded from GenBank (3 samples) with an accession number.

The length and weight relationship could be analyzed using the Linear Allometric Model (LAM) equation (Sparre and Venema, 1999) as follows.

$$W = (aL^b)$$

Note: W is fish weight (gram), L is fish length (cm), a is linear regression intercept, b is regression coefficient. Value of b from the results of this calculation can reflect fish growth patterns. If the value of b = 3, then the growth pattern is isometric or weight gain is equivalent to the growth in length and if the value is ≠ 3, then the growth pattern is allometric. Allometric growth patterns are divided into two, namely positive allometric and negative allometric. If the value of b under 3 is called negative allometric (the length increase is faster than the weight gain), and if the value of b above 3 is called a positive allometric (the weight gain is faster than the length increase).

Results

Molecular characteristic

Molecular characteristics of male bloating (*Rastrelliger kanagurta*) in a total of 9 samples (Primary), 3 (Secondary) and 1 (out group) were found 374 base pairs (bp) in the locus control region of the mitochondrial DNA region (Table 4). DNA electrophoresis showed the success of the Polymerase Chain Reaction (PCR) stage (Figure 2). The appearance and color of the band shows a positive sign of the results of the extraction and PCR stages. The selection and use of primers and specific reagent components are appropriate, helping the success of electrophoresis. The quality of DNA tissue has an influence on the success of DNA analysis stages.

Genetic diversity

The genetic diversity of *R. kanagurta* was high with a total number of haplotypes and diverse nucleotide diversity (Table 6). Genetic diversity (Hd) and nucleotide diversity in Bacan (Hd = 0.0666 and Π = 0.006), Morotai (Hd = 0.0668 and Π = 0.006), Ternate (Hd = 0.0667 and Π = 0.006) and secondary Indian data (Hd = 0.0667 and Π = 0.002) (Table 3).

The minimum spawning networks (MSN) found 5 haplotype networks from a total of 12 samples (Figure 3). The haplotype network showed that there were 2 specific haplotypes, 8 haplotypes mixed between locations and the same 2 haplotypes (Figure 3). The distribution of the haplotype network showed that there was a relationship between the haplotype network and did not indicate grouping. The results of the analysis, which found high genetic diversity and specific haplotypes, gave a profile of the genetic structure of mackerel (*R. kanagurta*) populations.

Morphology characteristic

Sample collection at three locations found male mackerel (*Rastrelliger kanagurta*). Morphological measurements of mackerel at three locations were found to be of various sizes (Table 6). Morphological measurements of standard length, head height, body width, pectoral fin length and tail were found to have varying mean values (Table 6). Such variations are due to the influence of age and sex.

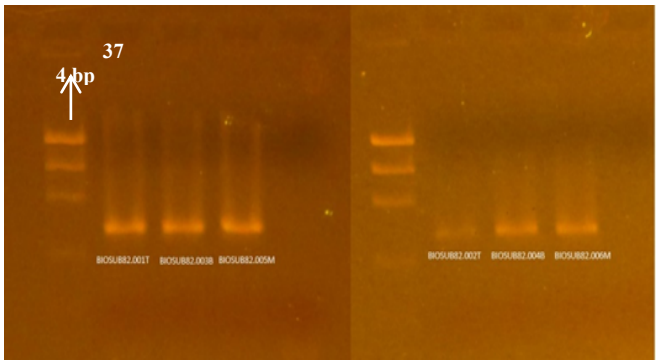

Figure 2. Elektroforesis DNA *Rastrelliger* Spp.

Table 1. Sample, number of samples, base length (bp), location and source

Name	Samples Total	Base pairs (bp)	Location	Sources
Rastrelliger kanagurta	9	374	North Maluku Sea	-
	342	1140	Strait of Malacca (SSOM), South China Sea (SCS), Sulu Sea and Celebes Sea	Akib *et al.* (2015)
	211	445	Jawa Sea	Indaryanto *et al.* (2015)
	24	-	East Indonesia Sea	Zamroni *et al.* (2017)
	30	154-220	Andaman Sea	Munpholsri *et al.* (2013)
	25	460	Thailand, Cambodia, Madagascar dan Vietnam	Kielpinski *et al.* (2014)
	53	1200	West coast of Peninsular Malaysia	Darlina *et al.* (2011)
	65	-	India	Jayasankar *et al.* (2004)

Table 2. Number of samples, base pairs, species and sources.

No	Samples Total	Base pairs (bp)	Species	Sources
1	9	374	*Rastrelliger kanagurta*	-
2	800	625	*Udang Sentadu*	Barber *et al.* (2006)
3	280	860	*Bigeye Tuna*	Chiang *et al.* (2008)
4	581	350	*Tuna Albacore*	Davies *et al.* (2011)
5	18	792	*Walking Shark*	Allen *et al.* (2013)
6	41	517	*Yellowfin Tuna*	Akbar *et al.* (2014)
7	582	654	*Shark*	Sembiring *et al.* (2014)
8	59	600	*Shark*	Prehadi *et al.* (2015)
9	39	526	*Epinephelus spp*	Jefri *et al.* (2015)
10	39	750	*Sarcophyton trocheliophorum*	Kusuma *et al.* (2016)
11	179	656	*Turbinidae*	Saleky *et al.* (2016)
12	72	512	*Yellowfin Tuna*	Aris *et al.* (2017)
13	30	546	*skipjack*	Akbar *et al.* (2018)
14	6	350	*Hemirhampus sp*	Achmad *et al.* (2019)

Table 3. Genetic diversity analysis.

Locations	*n*	H_n	H_d	Π	*Base Pairs*
Bacan	3	2	0.666	0.006	374
Morotai	3	2	0.668	0.006	
Ternate	3	2	0.667	0.006	
Indian	3	2	0.667	0.002	

n = number, Hn = Haplotype number, Hd= Haplotype diversity, ΠΙ = Nucleotide diversity

Table 4. Genetic diversity comparation between locations.

Locations	*n*	H_n	H_d	Π	*Base Pairs*
North Maluku (Bacan, Morotai, Ternate)	9	4	0,639	0,005	347
Indian_GenBank	3	2	0,0667	0,002	

n = number, Hn = Haplotype number, Hd= Haplotype diversity, ΠΙ = Nucleotide diversity

Table 5. Genetic diversity comparation research result and marine organism other.

Species	Genetic Diversity	Literature
R. kanagurta	0.666-0.668	-
R. kanagurta	0.972-1.000	Akib *et al.* (2015)
R. kanagurta	0.21-0.91	Munpholsri *et al.* (2013)
R. kanagurta	0.274-0.420	Zamroni *et al.* (2017)
R. brachysoma	0.000	Zamroni *et al.* (2008)
Cromileptes altivelis	0.774-0.794	Sembiring *et al.* (2013)
T. Crocea, T. Maxima dan T. Squamosa	0,480-1.000	De Boer *et al.* (2014)

Species	Genetic Diversity	Literature
Tripneustes gratilla	0.900	Toha *et al.* (2014)
Sarcophyton trocheliophorum	0.600-0.972	Kusuma *et al.* (2016)
Turbinidae	0.657-0.816	Saleky *et al.* (2016)
T. albacares	0.977-1.000	Aris *et al.* (2017)
Katsuwonus pelamis	0,800 – 0,995	Akbar and Labenua (2018)
T. obesus	0.996-1.000	Akbar *et al.* (2018)
Hemiscyllium halmahera	0.142-0.805	Madduppa *et al.* (2020)

Table 6. Morphological characteristics of male mackerel (*Rastrelliger kanagurta*).

No	Morphology Characters (cm)						
	Location	Standar Length	Head Height	Head Length	Body Width	Pectoral Fin	Tail
1	Bacan	29	05.01	07.08	06.03	03.03	05.09
		30	05.02	07.09	06.05	03.04	6
2	Morotai	28	5	07.08	6	03.03	6
		29	5	07.08	06.03	03.03	05.09
3	Ternate	29	05.01	8	06.01	03.03	6
		28	5	07.08	06.01	03.02	05.09
Everages		29	3	2	1	0	3

Table 7. Genetic diversity, population structure genetic and genetic distance catagories.

Analysis	Catagory			
	Low	Middle	High	Literature
Genetic diversity (H_d)	0.1-0.4	0.5-0.7	0.8-1.00	Nei, 1987
Amova population pairwaise (*Fst*)	0.1-0.3	0.4-0.7	0.8-1.00	Excoffier *et al*,1992
Genetic distance (*D*)	0.010-0.099	0.1-0.99	1.00-2.00	Nei, 1972

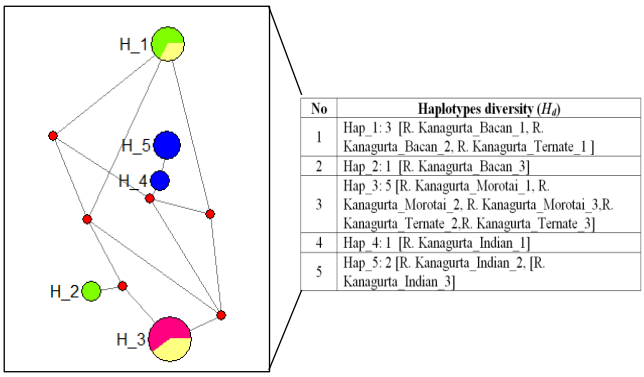

Figure 3. Haplotype distributions *R. kanagurta* using Network 5.0.1.1. Note. Green = Bacan, pink = Morotai, yellow = Ternate dan blue = Indian.

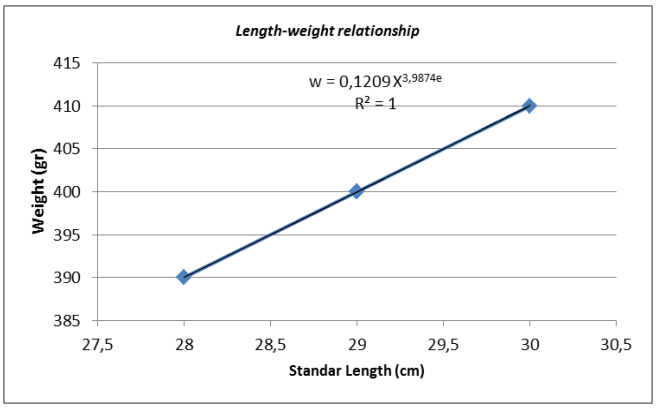

Figure 4. Length weight relationship *R. kanagurta*.

Fish populations have increased exploitation, sustainability strategies and sustainable use have to be taken into account so management and conservation are needed in this area. Calculation of mackerel (*R. kanagurta*) length and weight obtained by the coefficient of determination 1.00 which indicated that there was a weight gain of 100% which can be explained based on the regression other than the value of R2 greater than 1 which suggested a close relationship between the two variants.

Discussion

DNA isolation has an important role in the success of extraction of purification and quantity of DNA (Abdullah *et al.*, 2019). The size of base pairs (bp) was the normal size of sea fish sequences, where the length of the base size in fish ranges from 200-1500 bp (Liu *et al.*, 1999; Ali *et al.*, 2004). Fragment length reported by Jayasankar *et al.* (2004), Darlina *et al.* (2011), Munpholsri *et al.* (2013), Kielpinski *et al.* (2014), Akib *et al.* (2015), Indaryanto *et al.* (2015) and Zamroni *et al.* (2017) (Table 1). Fragment length (bp) was found to be short compared to other studies (Table 1). The difference in base pair (bp) is due to the use of primers, PCR processes, contamination of organic matter, number of samples, DNA quality, primary specifics, primary base composition, environment, food and offspring, but do not show the effect of sequencing results (Williams *et al.*, 1990 ;

Shizuka and Lyon, 2008, Nuryanto and Kochzius, 2009; Akbar *et al.,* 2014; Jefri *et al.,* 2015; Saleky *et al.,* 2016). The difference in base length can also be caused by the presence of nucleotide mutations and substitution in the genes of each species (Xio *et al.,* 2007; Akbar *et al.,* 2018). Length of DNA fragments of marine fish reported by Barber *et al.* (2006) found 625 bp in sentadu shrimp (stomatopoda), Chiang *et al.* (2008) with a length of 860 bp for large-eye tuna species, Davies *et al.* (2011) found 350 bp in albacore tuna, Allen *et al.* (2013) found 792 bp in walking sharks (*Hemiscyllium halmahera*), Akbar *et al.* (2014a) found 517 fragment length in yellow fin tuna, Prehadi et al. (2014) and Sembiring *et al.* (2015) obtained 600-700 bp from shark samples, Jefri *et al.* (2015) found 526 bp of the Epinephelus spp grouper species, Kusuma *et al.* (2016) i.e. 750 bp on *Sarcophyton trocheliophorum* soft corals, Saleky *et al.* (2016) obtained 656 bp in a turbinidae type gastropod, Aris *et al.* (2017) found 512 bp in yellow fin tuna, Akbar *et al.* (2018) obtained 546 bp in skipjack fish and Achmad *et al.* (2019) found 350 bp in Hemirhampus sp (Table 2). The difference in base pair in marine fish is due to variations in species, habitat and environmental adaptation (Martinez *et al.*, 2018; Shuai *et al.,* 2018). Pelagic fish are migratory and form different biological characteristics.

Comparison of *R.kanagurta's* genetic variability with other marine organisms such as *Cromileptes altivelis, T. Crocea, T. Maxima* and *T. Squamosa, Tripneustes gratilla, Sarcophyton trocheliophorum, Sarcophyton trocheliophorum, Turbinidae, T. Maxima* and *T. Squamosa, Tripneustes gratilla, Sarcophyton trocheliophorum, Sarcophyton trocheliophorum, Turbinidae, T. Maxima and T. Squamosa, T. obesus* and *Hemiscium deer* (Sembiring *et al.,* 2015; Kusuma *et al.,* 2016; Saleky *et al.,* 2016; Aris *et al.,* 2017; Akbar and Labenua, 2018; Akbar *et al.,* 2018; Madduppa et al. , 2020) showed high genetic quality. The ability to migrate leads to high inter-population encounters and genetic diversity (Zardoya *et al.,* 2004; Chiang *et al.,* 2006;2008; Akbar *et al.,* 2014; Akbar *et al.*, 2018). The overall value of *R. kanagurta*'s genetic diversity, when compared with other studies, was 0,000-1,000 (Table 10). Similarities and differences in the values of genetic diversity were due to differences in the number of samples used in the study (Akbar *et al.,* 2014a). Nei (1987) said that the genetic diversity value of one species depends on the sample size found. Avise *et al.* (1989) stated that the overall haplotype diversity for mtDNA for some fish is in the range of 0.473-0.998. According to the genetic diversity criteria (Nei, 1987), the results of the study found illustrating that mackerel (*R.kanagurta*) has normal genetic diversity (Table 7). Even though this species was a target of conservation in each water, but with the ability to migrate and produce the fast tuna population can survive. Genetic diversity gives the ability to adapt to changes in the environment and climate and disease and showed the species has a large population size (Zamroni *et al.,* 2017). is the main target species in fishing small pelagic fish categories, this is because this species is a fish that people consumption every day in North Maluku. High genetic diversity of mackerel (*Rastrelliger* sp.) reported by Akib *et al.* (2015) (Hd = 0.972-1000) in the Strait of Malacca (SOM), South China Sea (SCS), Sulu Sea and Celebes Sea, Munpholsri *et al.* (2013) (Hd = 0.21-0.91) in Andaman Sea, Thailand and Zamroni *et al.* (2017) (Hd = 0.274-0.420) in East Indonesia Sea (Table 4). Previous studies reported Zamroni *et al.* (2007) found low genetic diversity (Hd = 0,000) on the North coast of Java (Table 10). Zamroni *et al.* (2007) said that low genetic diversity was presumably that mackerel (*R. brachysoma*) originating from a population stock may also be spawning sites in the same area, this results in genetic introgression so that it showed low genetic diversity on the North coast of Java (Table 5).

Research on morphological characteristics in Indonesia reported by Telleng *et al.* (2010) in the waters of Buyat Bay, Suruwaky and Gunaisah (2013) Sorong sea waters, Tangke (2014) in the waters of North Maluku and Sonodihardjo and Yahya (2015) in the waters of Pancana, showed that there are no significant differences. *Rastrelliger* species have similar morphological characteristics despite different locations (Muchlisin *et al.,* 2009; Indaryanto *et al.,* 2015). Populations with high genetic closeness have morphological and genetic similarities as well as the environmental aspects (Kartika *et al.,* 2017). Therefore, morphological studies are important in terms of the management and conservation of fisheries that are adequate for biodiversity and sustainability (Indaryanto *et al.,* 2015). Morphological measurements of mackerel obtained in the standard size category. This illustrated that the mackerel population was still in normal condition, although the rate of catch utilization continues to increase. Zamroni et al. (2008) suggested that the existence of mackerel (*Rastrelliger* spp.) in the sea was captured using a mini purse seine in artisanal fisheries. Tangke (2014) suggested that mackerel in the waters of the island of Ternate has better growth than in the waters of the Java Sea, the Malacca Strait and the Jakarta Bay, allegedly related to the ecological factors of the habitat. The long weight relationship of fish needs to be known for the purposes of sustainable fisheries management. The management strategy is based on fish size data and information. Utilization rates that

are not proportional to population growth in nature cause problems. The exponential graph of mackerel showed that there was a close relationship between individual weight and length (Figure 4).

The calculation results showed that 5.2646 was obtained, thus indicating that the value of b> 3. The length of the fish's weight has a value of b = 3, indicating that the growth pattern was isometric or weight gain is equivalent to the growth of the fish's length (Fuadi *et al.,* 2016). Isometric growth patterns indicated that the population of mackerel (*Rastrelliger kanagurta*) in normal conditions. Although fishing operations were high so that it can threaten mackerel populations. The environment of sea on the islands of Morotai, Bacan and Ternate that has not been polluted (Marasabessy *et al.*, 2010; Edward, 2015; Najamuddin *et al.,* 2020), provides the opportunity for the presence of mackerel to make it a food area. Scientific approach was carried out such as reducing fishing effort in order to obtain maximum profit, but it still leads to fishing that is already large (Prahadina *et al.,* 2015). The results obtained were different from the Suruwaky and Gunaisah report (2015) which found that the resources of mackerel (*Rastrelliger kanagurta*) at Sorong sea waters have been over exploited so that allometric growth was negative. Tangke (2014) found mackerel caught in the coastal waters of Ternate Island has a fast growth rate and a high mortality rate, where generally caught by fishermen are mackerel size 14.90 which was the size of fish that have not yet performed spawning but it could also be seen that the rate of mackerel exploitation in Ternate island's coastal waters have more catch with an E value of 0.54. Male bloating fisheries in Barru Regency waters have experienced more capture (Sonodihardjo and Yahya, 2015). Indian mackerel (*Rastrelliger kanagurta*) in Thailand has experienced a decline in catch over the past few years and has been identified as overexploitation (Munpholsri *et al.,* 2013).

Conclusions

Molecular characteristics of male bloating (*R.kanagurta*) in a total of 9 samples (Primary), 3 (Secondary) and 1 (out group) were found 374 base pairs (bp) in the locus control region of the mitochondrial DNA region. The results of the study found to describe that mackerel (*R.kanagurta*) has normal genetic diversity. The minimum spawning networks (MSN) found 5 haplotype networks from a total of 12 samples. The distribution of haplotype networks showed that there was a relationship between haplotype networks and did not show grouping. High polymorphism increased the ability of populations in disturbed environments. Morphological measurements of standard length, head height, body width, pectoral fin length and tail were investigated to have varying mean values. Isometric growth patterns indicated that the population of mackerel (*R.kanagurta*) in normal conditions.

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