IOSR Journal of Agriculture and Veterinary Science (IOSR-JAVS)
ISSN: 2319-2380, ISBN: 2319-2372. Volume 1, Issue 6 (Nov. - Dec. 2012), PP 12-16
www.iosrjournals.org

The Association between Genetic Variations and Omega-3 Production on *Sardinella lemuru* in Lombok Strait

Mahrus[1, 4], Sutiman Bambang Sumitro[2], Nashi Widodo[2], Aida Sartimbul[3]
[1]*Doctoral Program, Faculty of Agriculture, Brawijaya University, Indonesia*
[2]*Biologi Department, Faculty of Mathematics and Natural Sciences, Brawijaya University, Indonesia*
[3]*Faculty of Fishery and Marine Sciences, Brawijaya University, Indonesia*
[4]*Biology Department, Faculty of Education and Teacher Training, University of Mataram, Indonesia*

Abstract: *Sardinella lemuru or Sardine is a marine fish that contains a high amount of omega-3 that is abundant in Lombok Strait. Therefore, the genetic variation of the S. Lemuru and its omega-3 production has not been widely studied yet. Furthermore, we have analyzed the correlation between genetic variation and omega-3 content in S. lemuru in Lombok strait. The variations of the S. lemuru were analyzed by using morphology and RAPD method. The result suggested that the S. lemuru in Lombok Strait has three variants that were associated with omega-3 production. Although differences of omega-3 content in marine fish could be caused by various factors, but the differences in the omega-3 content of Sardine in Lombok strait can be assumed due to genetic variations. This study also has discovered a molecular marker based on RAPD method by using primer 5'-AAGAGCCCG to identify Sardine that has high production of omega-3 cheaply, which is very beneficial for managing the conservation of Lombok Strait.*

Keywords: *Sardinella lemuru, omega-3, RAPD, Genetic Variations, Lombok strait.*

I. Background

Sardinella lemuru or Sardine is known as a small pelagic fish that contains a high amount of omega-3 and spreads in eastern Indian Ocean including southern coasts of East Java, Bali and Lombok. Lombok Strait has abundant and has several kinds of *S. lemuru*. It indicates that Lombok Strait is very fertile and appropriate for *S. lemuru* to live, making it a potential place to conserve the availability of main source of omega-3 in Indonesia. The characteristic of Lombok Strait is relatively narrow, not so deep, with upwelling that occurs annually [1], a suitable place for many kinds of fish, including *S. lemuru*, to live. Although it has several types, the genetic diversity of *S. Lemuru* in Lombok Strait has not been widely studied yet. Therefore, we have analyzed the genetic variations using molecular approach by RAPD method. Molecular approaches are strongly needed for understanding the relationships of many species[2, 3].

Omega-3 is beneficial to prevent coronary heart disease, diabetes, cancer, nervous system and eyes diseases [4-8]. Sardine contains the 3rd highest of omega-3 among other marine fish after Mackerel and Salmon [9]. The variation of omega-3 concentration in marine fish were caused by multifactors, including species and variant in species and food preference [10]. Furthermore, we have analyzed the association between genetic variation and omega-3 content on *S. lemuru* in Lombok Strait. The result suggested that the *S. lemuru* in Lombok Strait had three variants that were associated with omega-3 production. This finding is very important to provide basic information for fishery management and conservation in Lombok Strait.

II. Methodology

Collection and Identification of *S. lemuru*

A total 260 *S. lemuru* (80 g/fish) were collected from Lombok Strait and transported to the Laboratory of Biology, Mataram University, for further analysis. The *S. lemuru* population was caught from 5 locations in Lombok Strait during September-December 2010. Lombok Strait is located between Bali Island and Lombok Island, on 9º20′ – 6º20′ south latitude, 115º 30′ – 119º 30′ degrees east longitude. The *S. lemuru* were identified and grouped according to their body shapes, head shapes, types of scale, morphometric (i.e. standard length, head length, body width), and meristic characters (the total number of scutes, pre-pelvic scutes, post-pelvic scutes, dorsal fin rays, pelvic fin rays, anal fin rays, and branchiostegal rays).

PCR-RAPD

The genetic variations of the *S. lemuru* were analyzed by using RAPD method. The method is based on the ability of primers to amplify DNA by using Polymerase Chains Reaction (PCR). PCR was performed in 50 uL reaction volume consisting of PCR buffer, 25 mM $MgC1_2$, dNTPs, Primer, and 0.25U of Taq polymerase (Promega Corp), and 5 uL of DNA template. The primers used were four random primers; they are 5'-AACGCGAAC, 5'-AAGAGCCCG, 5'-AACGCCCAG, 5'-AAGAGCAGC. The conditions of amplification

were as follows: pre-denaturation 94^{o} C for 10 minutes, followed by denaturation for 30 sec in 94^{o} C, annealing in 36 ^{o}C for 30 seconds, and extension in 72^{o} C for 1 minute. The denaturation, annealing and extension were conducted on 35 cycles, respectively. Furthermore, the final extension was performed in 72^{o} C for 5 minutes. Amplification reaction was performed by using MyCycler machine (Biorad, USA). The PCR products were separated in 2% agarose gel electrophoresis by using Tris-borate buffer at a constant voltage 100 V for approximately 1 hour. DNA band were observed according to the intensity of Ethidium bromide under UV light exposure. The size of the PCR products were measured on the basis of DNA Marker (Invitrogen, USA). Gels were recorded on Polaroid film using the MP4 + Camera System (Polaroid). The genetic polymorphism was analyzed based on the presence or absence of DNA bands. The variation of the band indicated the occurrence of genetic polymorphism of the *S. lemuru*. The DNA profiles were used to construct a phylogenetic tree by using N-TSYS software.

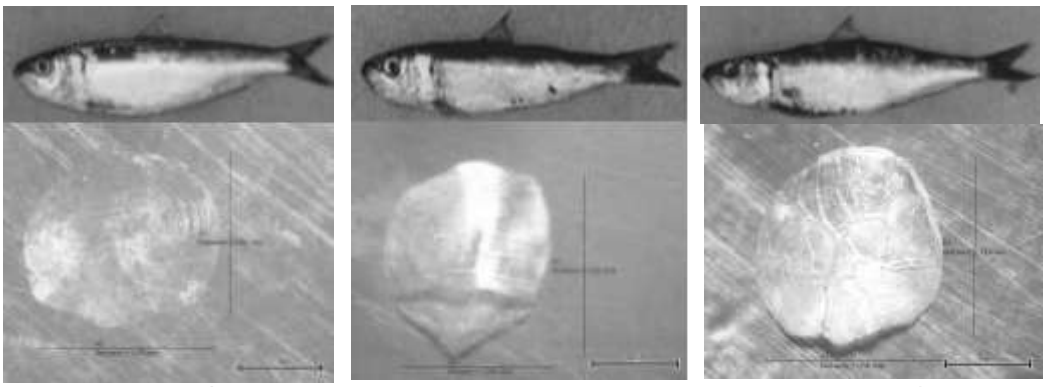
Extraction of Omega-3

The content of omega-3 fatty acid from *S. lemuru* was extracted by using urea inclusion method [11]. The meat of sardines was added by Aquades as much as 10 % of the sardines' weight, then boiled for 20 minutes. Fish stew was filtered to separate crude oil and to remove out debris. The crude oil was purified by adding NaCl
2.5 % of solution, then heating it at temperature 50^{o} C until oil crust was developed. The oil was separated from water with a separating funnel, then added by bentonite, mixed and stored for some minutes. After settling a few moments, the clean oil was collected by filtering, then storing it in a sealed container to avoid contamination from sun rays and air. As much as 350 g of fish oil were added by 700 g NaOH-EDTA solutions (120 g NaOH and 1.25 g of Ethylene Diamine Tetraacetic Acid (EDTA) were deluted with 400 ml Aquades and 400 ml of 96% ethanol). Saponification was conducted at room temperature for 8 hours while, at the same time, being constantly mixed and drained with nitrogen gas. The saponification products were adjusted by using 6 N HCl until pH 1, followed by adding 200 ml n-hexane, and dried by using Rotavapor on 30^{o}C. 25 g residues were diluted by 100 ml hot urea solution (65 - 70 ^{o}C) and 267 ml methanol, then mixed until forming limpid solution. The solution-urea complex was stored for one night until it formed crystal at -36^{o}C. Then the liquid phase was evaporated by using vacuum-evaporator at room temperature. The residues of evaporation product were diluted by 0.1 N HCl 125 ml and n-hexan 125 ml, then the hexane phase was separated. The residues were added by 50 ml n-hexane, mixed, then evaporated by vacuum evaporator. The residues were omega-3 which was, then, added by octyl gallate as stabilizer.

III. Discussion

Characteristics of Morphology of *S. lemuru*

According to the body shapes, head shapes, and patterns of scale, it was shown that the *S. lemuru* (260 fish) belonged to three groups, i.e. variant A (112 fish), variant B (80 fish), and variant C (68 fish) (Figure 1). The three variants had different body shapes, i.e. cylindrical for variant A, flattened-elongated for variant B, and flat up for variant C. The head shapes from the three variants were significantly different, i.e. rounded for variant A, rounded-elongated for variant B, and elongated for variant C. The *S. lemuru* generally had an average length of head which was shorter (26-29%) that the total length, ranging between 6.2 and 7.4 cm (mean: 6.6 cm). A previous study reported that Sardine from the northern Atlantic and Mediterranean area had smaller heads and head-to-body ratio than those from southern Iberia and northern Morocco [12].



Variant A Variant B Variant C

Figure 1. Variants and its Cycloid scales pattern of *S. lemuru* in Lombok Strait

Our analysis on the shapes of scales shows that all the three variants of *S. lemuru* has cycloid type of scales with various patterns, i.e. tiny and circle scale for Variant A, irregular pattern for Variant B, and regular

pattern for Variant C (Figure 1). The scale patterns are consistent with Bleeker's (1853) report [13]. The result indicates that *S. lemuru* in Lombok Strait has three morphology variants. Therefore, the variations should be elucidated based on morphometric and meristic characters.

Table 1. Morphometric characters of *S. lemuru* in Lombok Strait

Morphometric Characters	Variant A (cm)	Variant B (cm)	Variant C (cm)
Total length	22± 0.45	22± 0.5	22± 0.58
Body width	6.05±0.21	6.05±0.26	6.05±1.32
Head length	6.6± 0.35	6.6± 0.42	6.6± 0.5

Table 2. Meristic characters of *S. lemuru* in Lombok Strait

Meristic Characters	Variant A	Variant B	Variant C
Scutes number	34±0.45	34±0.51	34±0.57
Pre-pelvic scutes	21 ±0.25	22±0.45	23±0.65
Post-pelvic scutes	11.5±0.25	11.5±0.45	11.5±0.65
Dorsal fin rays	15±0.42	15±0.5	15±1.0
Pelvic fin rays	8±0.56	8±0.78	8±0.81
Anal fin rays	21±0.51	21±0.67	21±0.73
Branchiostegal rays	6±0.56	7±0.7	6±0.78

However, the morphometric (Table 1) and meristic characters (Table 2) from all of those fish are similar. The morphometric and meristic characters of the *S. lemuru* are as follows: the total length is 22 cm; the body shape is cylinder; the body width is less than 30 % of standard length; the number of pre-pelvic scutes is 23; the number of pelvic rays is 8 with 1 unbranched and 7 branched; and, the number of gillrakers is 77 and 6 branchiostegal rays. Other characters are the presence of black spot at hind border of gill cover and a faint golden spot behind the gill slits followed by a faint golden at midlateral line of the body. These characters are consistent with the previous study [13-15]. Moreover, Sardines in Lombok Strait have differences in size as compared to those in Sibolga bay, Indonesia [16], Mediterranean and Celtic sea [17], and from the north-eastern Atlantic and western Mediterranian [15]. Hence, the morphometric and meristic characters could not be used to identify variations of *S. lemuru* in Lombok Strait. Therefore, the three variations of *S. lemuru* based on its morphology (shape of head, body and scale) must be confirmed at the genetic level.

Molecular Marker and Phylogenetic Analyses

The analysis on the genetic variations by RAPD shows that a primer 5'-AAGAGCCCG is able to describe the differences between the three variations of *S. lemuru* in Lombok Strait (Figure 2a). The primer is adequate to amplify DNA in eleven different places of *S. lemuru* genome that can distinguish the three existing variants of the fish. Variant A is closely-related to variant C with similarity value 0.714; hence, the similarity value between A or C with B is 0.357 (Figure 2b). This indicates that the genetic variations of the fish are still high, and this information is very important to develop a concept for conserving *S. lemuru* as the main sources of omega-3 in Lombok Strait. Also, the data is similar to the condition of Sardines in India and Philippine [17, 18]. Furthermore, the primer is warrant for molecular markers to identify variants of *S. lemuru* in Lombok Strait.

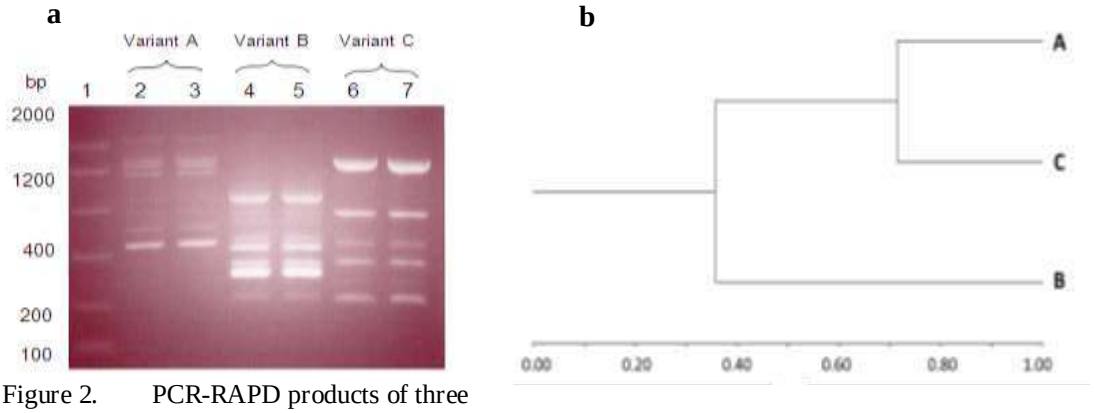


Figure 2. PCR-RAPD products of three variants of *S. lemuru* **(a)**. Number 1 is DNA standard; number 2 and 3 are variant A; 4 and 5 are variant B; 6 and 7 are variant C of *S. lemuru* on 2% gel Agarosa. The phylogenetic tree of *S. Lemuru* in Lombok Strait **(B).**

Content of Omega-3 Associated with Genetic Variant

The omega-3 content of the three variants of *S. lemuru* are not the same, ranging from 23.5% - 30.6%. The omega-3 concentrations in variant A, B and C are 30.6%, 23.5% and 26.7%, respectively. This data is very interesting because it shows an association between the levels of omega-3 to the genetic variant among the fish. Variant A contains the highest concentration of omega-3 compared to the other two variants. Moreover, *S. lemuru* in Lombok Strait have higher omega-3 content compared to Sardines in Bali Strait (18-23.3%) [11]. The other report presents that generally Sardine contains omega-3 ranging from11.33% - 40.26% [19-23].

Differences in omega-3 products from marine fish are caused by various factors, i.e. food consumed by the fish [8, 24-26], genetics, and environmental characteristics [27]. Nonetheless, the study about omega-3 concentration on *Gadus morhua* in Atlantic is not correlated with food consumed [28]. Another report explains that omega-3 concentration corresponds with Δ6-Desaturase activity in Zebrafish [3]. Since the three variations of the fish were found at the same location and the same season, the different content of omega-3 in Sardine in Lombok Strait can be assumed to be due to genetic variation. Taken together the result, it is concluded that *S. lemuru* in Lombok Strait has three variants that are associated with the omega-3 production. The variations could be identified by using the simple RAPD by employing one primer. This study has discovered a molecular marker to identify Sardines that have high omega-3 production easily and cheaply which is beneficial to the management of Lombok Strait.

IV. Conclusion

S. lemuru population in Lombok strait has three genetic variations that are associated with the omega-3 production. The genetic variations were identified based on RAPD method by using primer 5'-AAGAGCCCG.

Acknowledgements

The authors would like to thank the Directorate General of Higher Education, Ministry of Education and Culture of Indonesia for providing funding to carry out this research.

References

[1]. I Gede Hendrawan, Koji Asai. Numerical Study of Tidal Upwelling Over The Sill In The Lombok Strait (Indonesia). In: *Proceedings of the Twenty-first (2011) International Offshore and Polar Engineering Conference*. Maui, Hawaii, USA: the International Society of Offshore and Polar Engineers (ISOPE); 2011:949–956.

[2]. Marshall HD, Johnstone KA, Carr SM. Species-specific oligonucleotides and multiplex PCR for forensic discrimination of two species of scallops, Placopecten magellanicus and Chlamys islandica. *Forensic Sci. Int*. 2007;167(1):1–7.

[3]. Silva CA, Lima RCA, Teixeira AS. Isoenzyme electrophoretic patterns in discus fish (Symphysodon aequifasciatus Pellegrin, 1904 and Symphysodon discus Heckel, 1840) from the Central Amazon. *Genet. Mol. Res*. 2008;7(3):791–805.

[4]. Kris-Etherton PM, Harris WS, Appel LJ. Fish consumption, fish oil, omega-3 fatty acids, and cardiovascular disease. *Circulation*. 2002;106(21):2747–2757.

[5]. Nakamura MT, Nara TY. Structure, function, and dietary regulation of delta6, delta5, and delta9 desaturases. *Annu. Rev. Nutr*. 2004;24:345–376.

[6]. Hastings N, Agaba M, Tocher DR, et al. A vertebrate fatty acid desaturase with Δ5 and Δ6 activities. *Proc Natl Acad Sci U S A*. 2001;98(25):14304–14309.

[7]. Khoddami, A, A.A. Ariffin, J. Bakar and H.M. Ghazali. Fatty acid profile of the oil extracted from fish waste (head, intestine and liver) (Sardinella lemuru). *World Applied Sciences Journal*. 2009;7(1):127–131.

[8]. Sayanova O, Haslam RP, Calerón MV, et al. Identification and functional characterisation of genes encoding the omega-3 polyunsaturated fatty acid biosynthetic pathway from the coccolithophore Emiliania huxleyi. *Phytochemistry*. 2011;72(7):594–600.

[9]. Rubio-Rodríguez N, Beltrán S, Jaime I, et al. Production of omega-3 polyunsaturated fatty acid concentrates: A review. *Innovative Food Science & Emerging Technologies*. 2010;11(1):1–12.

[10]. Tu WC, Cook-Johnson RJ, James MJ, Mühlhäusler BS, Gibson RA. Omega-3 long chain fatty acid synthesis is regulated more by substrate levels than gene expression. *Prostaglandins Leukot. Essent. Fatty Acids*. 2010;83(2):61–68.

[11]. Abdullah Rasyid. Isolasi asam lemak tak jenuh majemuk omega-3I dari ikan lemuru (Sardinella sp). In: *Prosiding Seminar Riptek Kelautan Nasional*. Jakarta: . Pusat Penelitian Oseanografi LIPI, Jakarta; 2001:9–12.

[12]. Silva A. Morphometric variation among sardine (Sardina pilchardus) populations from the northeastern Atlantic and the western Mediterranean. *ICES Journal of Marine Science*. 2003;3139(03):1352–1360

[13]. Whitehead PJP. *Clupeoid Fish of the World (suborder Clupeoidei): Engraulididae*. Food & Agriculture Org.

[14]. Moyle PB, Cech JJ. *Fish: An Introduction to Ichthyology*. 5th ed. Benjamin Cummings; 2003.

[15]. Hall D, Beer A-J. *The Illustrated Guide to Marine Fish of The World: A Visual Directory of Sea Life Featuring Over 700 Fabulous Illustrations*. Ill. Anness; 2008.

[16]. Riama Verawaty Tampubolon, Sutrisno Sukimin, dan M. F. Rahardjo. Aspek Biologi Reproduksi Dan Pertumbuhan Ikan Lemuru (Sardirtella longiceps C.V.) Di Perairan Teluk Sibolga (Reproductive Biology and Growth of Sardine (Sardinella longiceps C.Y.) in Sibolga Bay). *.lurnol Iktiologi lndortesia*. 2002;2(1):1–7.

[17]. Maria R. Menezes. Little Genetic Variation in the Oil Sardine, Sardinella longiceps Val., from the Western Coast of India. *Aust. J. Mar. Freshwater Res*. 1994;45:257–64.

[18]. Samonte IE, Pagulayan RC, Mayer WE. Molecular phylogeny of Philippine freshwater sardines based on mitochondrial DNA analysis. *J. Hered*. 2000;91(3):247–253.

[19]. A. Khoddami, A.A. Ariffin, J. Bakar and H.M. Ghazali. Fatty Acid Profile of the Oil Extracted from Fish Waste (Head, Intestine and Liver) (*Sardinella lemuru*). *World Applied Sciences Journal*. 2009;7(1):127–131.

[20]. Okada T, Morrissey M t. Production of n-3 Polyunsaturated Fatty Acid Concentrate from Sardine Oil by Immobilized Candida

rugosa Lipase. *Journal of Food Science*. 2008;73(3):C146–C150.
[21]. Chakraborty K, Paulraj R. Selective enrichment of n−3 polyunsaturated fatty acids with C18–C20 acyl chain length from sardine oil using Pseudomonas fluorescens MTCC 2421 lipase. *Food Chemistry*. 2009. Available at: http://www.elsevier.com/locate/foodchem. Accessed July 5, 2012.
[22]. Patrícia de Oliveira CARVALHO, Paula Renata Bueno CAMPOS, Maximiliano D'Addio NOFFS, Deborah Helena Markowicz BASTOS & Joaquim Gilberto de OLIVEIRA. Enzymic Enhancement of ω3 Polyunsaturated Fatty Acids Content in Brazilian Sardine Oil. *Acta Farm. Bonaerense*. 2002;21(2):85–88.
[23]. Marion L´etisse, Murielle Rozi`eres, Abel Hiol, Michelle Sergent , Louis Comeau. Enrichment of EPA and DHA from sardine by supercritical fluid extraction without organic modifier I. Optimization of extraction conditions. *J. of Supercritical Fluids*. 2006;38:27–36.
[24]. James Henderson R, Tocher DR. The lipid composition and biochemistry of freshwater fish. *Progress in Lipid Research*. 1987;26(4):281–347.
[25]. Orban E, Lena GD, Nevigato T, et al. Quality Characteristics of Sea Bass Intensively Reared and from Lagoon as Affected by Growth Conditions and the Aquatic Environment. *Journal of Food Science*. 2002;67(2):542–546.
[26]. M.A. Hossain. Fish as Source of n-3 Polyunsaturated Fatty Acids (PUFAs), Which One is Better-Farmed or Wild? *Advance Journal of Food Science and Technology*. 2011;3(6):455–466.
[27]. Pigott GM, Tucker BW. *Seafood: Effects of Technology on Nutrition*. M. Dekker; 1990.
[28]. M. A. Mahar, Z. A. Larik, N.T. Narejo and S.I.H. Jafri. Limnological Study of Fishponds and Kalribaghar Lower Canal at Chilya Fish Hatchery Thatta, Sindh, Pakistan. *Pakistan J. Zool.* 210AD;42(4):419–430