GENETIC VARIABILITY OF THREE POPULATIONS OF FLYING FISH, *Hirundichthy oxycephalus* FROM MAKASSAR STRAIT

Andi Parenrengi[*)], Andi Tenriulo[*)], and Syamsul Alam Ali[**)]

[*)] *Research and Development Institute for Coastal Aquaculture, Maros*
[**)] *Faculty of Marine Science and Fisheries, Hasanuddin University, Makassar*

(*Received 30 November 2011 ; Accepted 12 May 2012*)

ABSTRACT

Flying fish, *Hirundichthy oxycephalus* is one of economically important marine species to Indonesia, particularly in Makassar Strait and Flores Sea. However, there is a limited published data on genetic variation in molecular marker level of this species. Random Amplified Polymorphic DNA (RAPD) was employed in this study to determine the genetic variability of three populations of flying fish collected from Takalar, Pare-Pare, and Majene in Makassar Strait. Genomic DNA was isolated from preserved muscle tissue using phenol-chloroform technique. Two selected arbitrary primers (CA-01 and P-40) were performed to generate RAPD finger printing of flying fish populations. The two primers generated a total of 81 fragments (loci) and 50 polymorphic fragments with size ranging from 125 to 1,250 bp. There were no significant differences in number of fragment and number of polymorphic fragment among populations. The high polymorphism (63.5±7.4%) was obtained from Takalar population followed by Pare-Pare (58.3±19.6%) and Majene population (57.7±0.8%). Similarity index of individuals was 0.60±0.17 for Takalar, 0.63±0.17 for Majene and 0.75±0.21 for Pare-Pare population. Seven fragments were identified as species-specific markers of *H. oxycephalus*. The UPGMA cluster analysis showed that the Takalar population was genetically closer to Pare-Pare population (D = 0.0812) than to Majene population (D = 0.1873).

KEYWORDS: flying fish, genetic variability, Makassar Strait, RAPD

INTRODUCTION

Flying fish, *Hirundichthy oxycephalus* Bleeker is one of important species in South Sulawesi waters, especially in Makassar Strait and Flores Sea. Flying fish *H. oxycephalus* was initially reported as *Cypcelurusoxy cephalus* in several previous studies. This species is familiar to the local coastal communities as one of fish protein sources and its highly valued eggs for export. On the contrary, the species wild stock has been left unmanaged and tends to show signs of overfishing, indicated by the decrease of population, abundance, and catch per unit of effort (CPUE) (Nessa*et et al*., 1993; Ali, 2005). Other indications of its population stress are showed by the changing of biological reproduction such the decrease of body length, increase the fecundity but decrease the egg diameter, and earlier spawning period (Ali, 2005). A good management strategy and the initiation of breeding program of the flying fish perhaps can be suggested as the solutions of these ever growing problems. For that reason, the genetic data of this species is very important as a baseline data for its future management.

Corresponding author. Research and Development Institute for Coastal Aquaculture
Jl. Makmur Dg. Sitakka No. 129, Maros 90512, Sulawesi Selatan, Indonesia. Tel.: +62 411 371544
E-mail address: andi_parenrengi@hotmail.com

Genetic variation is an important feature of a population not only for short-term fitness of individuals but also for long-term survival of the population by which it allows adaptation of fish to a changing environmental condition. Genetic diversity is also similarly important for farmed population which allows selective breeding and preventing loss of fitness due to inbreeding depression. Genetic variability can be determined by morphological characters (morphometric analysis), allozyme electrophoresis (protein pattern), and DNA fingerprinting. The genetic variations of four wing samples of flying fish *H. affinis* have been studied at molecular DNA level (Gomes *et al*., 1998; 2000). The significant morphological differentiation of flying fish *H. oxycephalus* has been revealed by morphometric analysis from several populations in Makassar Strait and Flores Sea (Ali, 2005). Currently, DNA fingerprinting technique is extremely efficient for detection of molecular genetic markers that may be utilized in assessment of genetic variation in fish, differentiation of stocks or populations and fisheries management. In more recent development in the detection of genetic polymorphisms is random amplified polymorphic DNA (RAPD). This technique, which is based on the polymerase chain reaction (PCR), amplifies random genomic segments with a single oligonucleotide primer of arbitrary sequence (Williams*et et al*., 1990). In contrast to isozymes, RAPD provides a more arbitrary sample of the genome and generates essentially unlimited numbers of loci for use in genetic analysis (Fritsch & Rieseberg, 1996). Genetic differentiation based on RAPD analysis in various fish species has been noted in many studies (Bielawski & Pumo, 1997; Coccone *et al*., 1997; Koh *et al*., 1999; Liu *et al*., 1999; Imron *et al*., 2009; Mulyasari *et al*., 2010, Moria *et al*., 2010; Imron *et al*., 2010; Iskandariah *et al*., 2011; Lante *et al*., 2011; Nugroho *et al*., 2011). But so far, no published data on genetic variation of the populations of flying fish *H. oxycephalus* from Makassar Strait, Indonesia.

The present study was aimed to examine the usefulness of RAPD analysis in detecting genetic variability of flying fish *H. oxycephalus* populations sampled from Makassar Strait, Indonesia. The obtained data will be a useful information in determining breeding program and genetic improvement as well as management and conservation strategies of flying fish.

MATERIALS AND METHODS

Collection of Samples

The flying fish *H. oxycephalus* samples (N = 15) were collected from Makassar Strait (Takalar, Pare-Pare, and Majene), Indonesia. The range of body weight and total length of samples were 55.39-59.42 g and 195.92- 202.27 mm, respectively. Approximately 50 mg of fresh muscle tissue from each individual was preserved in TNES-Urea buffer (6 M urea; 10 mMTris-HCl; 125 mMNaCl; 10 mM EDTA; and 1% SDS, at pH 7.5) (Asahida *et al*., 1996). The preserved samples were transported at ambient temperature from the field and kept at room temperature in the laboratory prior to DNA extraction.

Figure 1. Locations of flying fish *H. oxycephalus* sampling sites

DNA Extraction

A phenol-chloroform technique was employed to isolate genomic DNA from the preserved muscle tissue of flying fish based on the method that has been developed for grouper fish (Parenrengi, 2006). Five hundred microlitres of lysis buffer (0.5 M NaCl, 0.001 M

EDTA, 1% (w/v) SDS, 0.8% (v/v) Triton X-100, and 0.1 M Tris-HCl; at pH 9.0) were added to the preserved muscle tissue in a 1.5 mL microcentrifuge tube and followed by addition of 40 mL of 10% (w/v) SDS and 40 mL of proteinase K (20 mg/mL solution). The samples were incubated at 55ºC for 1-3 hours (until completely lysed). The samples were treated with 25 mL of RNAase (20 mg/mL solution) and left at room temperature for 15-30 minutes. The samples were treated with 500-600 mL of phenol:chloroform:isoamyl alcohol (25:24:1) and then gently vortexed to homogenize. The samples were left at room temperature for 10 minutes before centrifugation at 13,000 rpm for 4 minutes. The top aqueous layer was removed to a new 1.5-mL microcentrifuge tube. The step of adding phenol:chloroform:isoamyl alcohol (25:24:1) was repeated twice. Samples were treated with one volume of chloroform: isoamyl alcohol (24:1) and centrifuged at 13,000 rpm for 2 minutes. Two volumes of ice-cold absolute ethanol were mixed to the upper aqueous layer by rapid inversion of the tubes several times. Precipitated DNA was collected at the bottom of the tubes as a white pellet after centrifugation at 6,000 rpm for 30 minutes. The pellet was washed with 1 mL of 70% ethanol and then centrifuged at 6,000 rpm for 15 minutes. The DNA was allowed to dry at room temperature and then resuspended with TE buffer (10 mMTris and 1 mM EDTA, pH 8.0). The genomic DNA was electrophoresed at a 0.8% (w/v) horizontal agarose gel at 55 volts for 1-2 hours in 1 x TBE buffer (0.9 M Tris, 1.1 M Boric acid and 25 mM EDTA at pH 8.3 for 10X) and the staining was done in 0.5 µg/mL of ethidium bromide for 20-30 minutes and following washing with distilled water for 5-10 minutes.The purity of genomic DNA obtained was estimated using a Spectrophotometer. Two approaches were used in this study to analyze the purity of genomic DNA. First, the DNA purity was quantitatively estimated from the ratio between the reading of absorbency at 260 nm and 280 nm (OD_{260}/OD_{280}) (Linacero *et al.*, 1998). Second, the DNA purity was qualitatively observed through the appearance of a single band on the gel.

DNA Amplification

Amplification reactions were performed in 25 mL volumes. Each reaction mixture contained 1X PCR buffer; 3.5 mM of $MgCl_2$; 0.4 mM of dNTPs mix; 0.4 M of primer; 2.0 units of taq DNA polymerase; and 50 ng of genomic DNA. RAPD primers applied in this study were CA-01 (5'-ttttttagccttttttgagc-3') and P-40 (5'-gttttcccagtcacgaggttgta-3'). The genomic DNA was amplified using a GeneAmp PCR system 2700 (Applied Biosystems) which were programmed at 45 cycles for 30 seconds of denaturation at 94ºC, 30 seconds of annealing temperature at 36ºC, 1 minute of primers extension at 72ºC, and a final extension of 2 minutes at 72ºC. Preparation of PCR mixture was always conducted in a laminar airflow cabinet in order to avoid contamination. The negative control, PCR amplification without genomic DNA, was done for every master mix PCR to ensure the contamination of PCR reactions. No amplification product in negative control indicates that the PCR products are not contaminated. A mixture of 7.0 mL PCR product and 2.5 mL loading dye was run on a 2.0% agarose gel electrophoresis at 55 volts in 1XTBE for 2-3 hours and then stained with 0.5 mg/mL of ethidium bromide for 20-30 minutes. The gel was washed with distilled water for 5-10 minutes prior to photographing with gel documentation (Biometra).

Data Analysis

The molecular weight of fragments was estimated based on the standard of DNA banding pattern from 100 bp DNA marker. The fragment that was present for all individuals in three populations of flying fish was considered as a species-specific marker. The fragments were valued as polymorphic when they were absent in some samples (but changes in banding intensity were not valued as polymorphic). Presence of fragment was scored as 1 while absence was scored as 2 at a particular position or distance migrated on the gel. A data matrix of 1's and 2's was entered into the data analysis package. Data analysis was performed using the program Tool for Population Genetic Analyses (TFPGA) Version 1.3 (Miller, 1997). The genetic similarity index was calculated across all possible pairwise comparisons of individuals using the formula: $S_{xy} = 2n_{xy}/n_x + n_y$ (Nei & Li, 1979). Where n_{xy} is number of fragments shared by individual x and y; n_x and n_y are the number of fragments scored for each individual. The dendrogram was constructed using Unweighted Pair-Group Method of Aritmethic (UPGMA) from TFPGA program. Number of fragments and polymorphic fragments of flying fish were analyzed by ANOVA from

Statistic Version 3.0. When ANOVA identified differences, multiple comparisons among means were made with least significant difference (LSD) program. Statistical significance was determined by setting the aggregate type at 5% for each set of comparisons.

RESULTS AND DISCUSSION

RAPD Profile

The present study showed that different primers generated different RAPD profiles from DNA amplification of flying fish genome. The total number of fragments and polymorphic fragments of flying fish generated from the two primers were 9-16 and 4-13 fragments, respectively; and their size wereranging from 125 to 1,250 bp (Table 1). The RAPD fingerprintings of flying fish generated by primer CA-01 and P-40 were shown in Figure 2.

The total fragment, generated by primer P-40 showed a higher number compared with primer CA-01. Variation in the fragment number and size range of *H. oxycephalus* generated by different primers within various populations suggest that thedifference of obtained RAPD profilesis causing the difference in the fragment profile generated by various primers. Some authors reported the number of fragment from different fish species employing the RAPD technique. Six to seventeen fragments were observed in tilapia, *Oreochromis* spp. (Bardakci & Skibinski, 1994); 1-6 fragments in orange roughy, *Haplostenthus atlanticus* (Smith *et al*., 1997); 5-8 fragments in *Salmo* spp. (Elo *et al*., 1997); 5-16 fragments in striped bass, *Morones axatilis* (Bielawski & Pumo, 1997); and 1-10 fragments in channel catfish, *Ictalurus* spp. (Liu *et al.*, 1999). Different fragment size generated by RAPD technique was also noticed in different species. For instance, the fragment size of 300-2,500 bp was detected in *Anguilla* spp. (Takagi &Taniguchi, 1995); 600-2,800 bp in orange roughy, *H. atlanticus* (Smith *et al*., 1997); 220-1,270 bp in *Salmo* spp. (Elo *et al*., 1997); 160-350 bp in striped bass, *M. saxatilis* (Bielawski & Pumo, 1997); and 200-1,500 bp in channel catfish, *Ictalurus* spp. (Liu *et al*., 1999).

Genetic variability is usuallly reflected in the polymorphism measurement in term of variation of DNA fragment profile.The highest polymorphism (63.6±7.4) of flying fish was obtained from Takalar population followed by Pare-Pare (58.3±19.3) and Majene population (57.7±0.8), however, the statistical analysis showed that the number and polymorphic fragment were not significantly different ($P>0.05$) among populations (Table 2). A high polymorphic fragment of all populations indicates a low level of inbreeding among individuals of each population. Bowditch *et al.* (1993) noted that polymorphism determines the relatedness of the group or the taxa; parentage analysis for domestic and wild animal species; identification of individuals for captive breeding program especially for endangered species; comparison of the wild and the cultivated species; and estimation of the inbreeding or outbreeding level within populations.

The high polymorphism (more than 50%) was obtained from all population in this study. This result confirms that the flying fish samples were collected from wild population. High polymorphism, detected with RAPD markers, is prob-

Table 1. Total number of fragments, total number of polymorphic fragments, and proportion of polymorphism, and size range of fragments of flying fish in different populations

Populations	Primers	Total number of fragments	Number of polymorphic fragments	Proportion of polymorphism (%)	Size range of fragments (bp)
Takalar	CA-01	12	7	58.3	350-1,250
	P-40	16	11	68.8	125-1,250
Pare-Pare	CA-01	9	4	44.4	400-1,250
	P-40	18	13	72.2	125-1,250
Majene	CA-01	12	7	58.4	350-1,200
	P-40	14	8	57.1	125-1,175

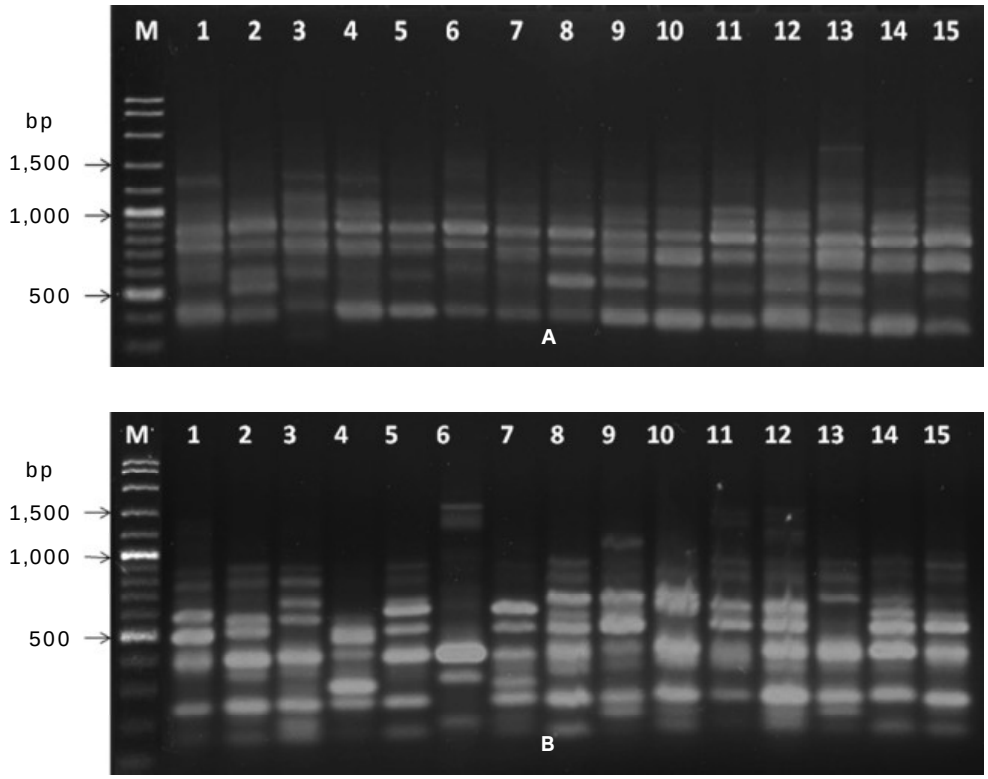

Figure 2. RAPD fingerprintings of flying fish *H. oxycephalus* generated by primer Ca-01 (A) and P-40 (B). M = 100 bp DNA marker, sample fish population of Takalar (lane 1-5), Pare-Pare (lane 6-10), and Majene (lane 11-15)

Table 2. Summary of genetic variability of flying fish from different locations revealed by RAPD analysis

	Populations		
	Takalar	**Pare-Pare**	**Majene**
Total number of primer	2	2	2
Total number of fragment	28	27	26
Number of fragment	14.0[a]±2.8	13.5[a]±6.4	13.0[a]±1.4
Number of polymorphic fragment	9	8.5	7.5
Polymorphism (%)	63.6[a]±7.4	58.3[a]±19.3	57.7[a]±0.8
Similarity index	0.60±0.17	0.75±0.21	0.63±0.17
Size range of fragments (bp)	125-1,250	125-1,250	125-1,200

Values in a same row followed by the same superscript are not significantly different (P>0.05)

ably due to the preferential amplification of the non-coding repetitive regions of the genome. Since primer is random in nature, coding and non-coding regions may be targeted of PCR amplification. Lynch & Milligan (1994) noted that the RAPD technique is expected to scan the genome more randomly than conventional method.

Several studies have reported that the RAPD marker is a useful method in detecting the polymorphism in different species and locations. A level of diversity ranging from 33.33%-50.00% was reported in the population of the pampean freshwater shrimp, *Macrobranchium borellii* with RAPD marker (D'Amato & Corach, 1996). Garcia & Benzie (1995) showed a high polymorphism of shrimp (39%-77%) occured in both RAPD marker and allozyme marker. Similar findings were also detected in the tilapia, *O. niloticus* population in Lake Albert and Lake Edward (Mwanja *et al*., 1996) and in striped bass, *M. saxatilis* from five populations (Bielawski & Pumo, 1997). The RAPD analysis has also shown the high polymorphisms (85.5%-98.5%) among individuals of sea cucumber within the same locality (Norazila, 2000). RAPD fingerprinting of six populations of giant freshwater prawn *M. rosenbergii* was reported with polymorphisms of DNA fragment ranging from 29%-76% (Imron *et al*., 2009), nilem fish *Osteochilus hasselti* from six populations with level polymorphism of 40%-68% (Mulyasari *et al*., 2010). While, the low level of polymorphism (18.2%-42.4%) was detected by RAPD marker in tilapia produced from cross-breeding between strains of tilapia (Iskandariah *et al*., 2011).

Similarity Index

The similarity index of intra population flying fish collected from different locations was 0.63±0.17 for Majene, 0.75.6±0.21 for Pare-Pare, 0.60±0.17 for Takalar (Table 2). The low similarity index obtained in the present study indicates the high degree of variability. Takalar population showed the highest variability while the Pare-Pare population had the lowest variability between individuals in the population. The high similarity indices of intra-population suggest the genetically closely related individuals in each population. This also indicates that the fish samples were collected from the relatively small geographic area for each population. The greatest amount of variation within individuals in Takalar population was also confirmed by the high polymorphism level among individuals (63.6%). The intra-population similarity index of Malaysian river catfish *M. numerus* ranged from 0.51 to 0.90 (Kim, 1998; Lim, 1998). Their study revealed unusual genotypes (5.04%-8.55%) which decreased the value of the similarity index. This also affected to the difference in the genetic distance in each population. Similar finding was reported from different species and populations. The similarity index between individuals among different populations of striped bass, *M. saxatilis* ranged from 0.92 to 0.96 (Bielawski & Pumo, 1997). The mean similarity index of the common silverbiddy, *Gerresoyena* was 0.558±0.060 within the Miyazaki population and 0.634±0.86 within the Okinawa population (Miyanohara *et al*., 1999). Parenrengi & Tenriulo (2008) reported that the similarity index of individuals of grouper fish from different locations was 0.86±0.07 for Pare-Pare, 0.80±0.11 for Makassar and 0.82±0.07 for Bone population, and also different species of groupers (*Epinephlus* spp.) was 0.62±0.07 for *E. areolatus*, 0.58±0.11 for *E. merra*, and 0.80±0.11 for *E. suillus* (Parenrengi, 2006).

Genetic Distance

The genetic distance obtained at this study varied from 0.0812 to 0.1873 in pair wise comparison between flying fish populations (Table 3). In the present study, the three populations were assessed their similarity based on the presence and absence of the marker phenotype that were interpreted as the genetic distance. This approach is commonly used in the analysis of dominant marker such as a RAPD technique. Genetic distance is presented as a dendrogram clustered by UPGMA method in which data are scanned initially for the smallest genetic distance and subsequently continued to scan the biggest one. Interestingly, the genetic distances of grouper fish at different populations (0.20-0.24) (Parenrengi & Tenriulo, 2008) and different species within genus of *Epinephelus* (0.52-0.67) (Parenrengi, 2006) were reported to be higher compared with this present study.

The dendrogram shows that the Takalar population was found to be genetically closer to Pare-Pare population (D = 0.08) than to Majene population. (D= 0.16) (Table 3 and

Table 3. Genetic distance of RAPD markers in three populations of flying fish

	Takalar	Pare-Pare	Majene
Takalar	0		
Pare-Pare	0.0812	0	
Majene	0.1873	0. 1280	0

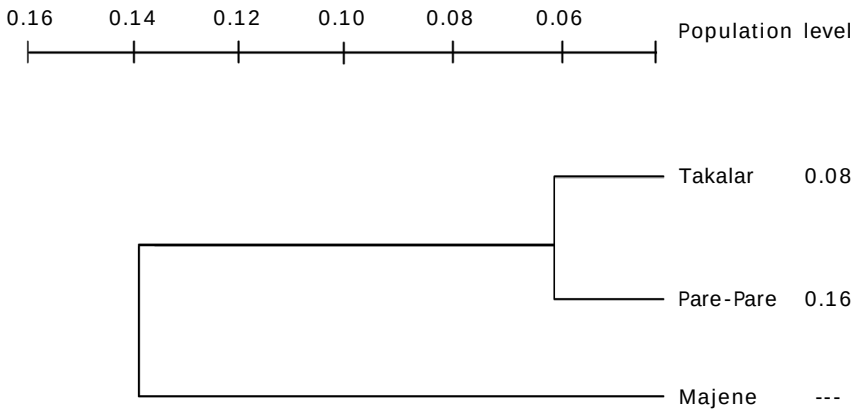

Figure 3. UPGMA cluster analysis based on the genetic distance generated from Nei and Li's indices at different flying fish populations

Figure 3). The results indicate that geographical factor influenced the genetic distance of the flying fish populations. The important factors affecting gene differentiation among populations are the isolation and the fish genetic drift (gene migration). The genetic drift is the random intergenerational change in gene frequency due to its finite population size (Jorde, 1995). Ferguson *et al*. (1995) also noted that in small isolated populations, genetic variability could be substantially reduced through genetic drift resulting in the loss of alleles and decline in heterozygosity. It was commonly agreed that since the sampling sites of populations were from neighboring sites located on the Makassar Strait, Sulawesi Waters, Indonesia, therefore, they might have originated from the same ancestral population and a close genetic relationship was expected. This also suggests that there is frequent migration or gene flow between these populations. Jorde (1995) explained that the gene flow reduces the genetic distance among populations; when two populations manifest the same genetic distance, they are expected to be communicated from one to another via gene flow. The value of genetic distance of flying fish populations revealed by RAPD analysis is relatively similar with genetic populations studies on the tilapia (0.04-0.034) (Bardakci & Skibinski, 1994) and hilsa sad populations (0.08-0.16) (Dahle *et al*., 1997), grouper (0.20-0.24) (Parenrengi & Tenriulo, 2008), giant freshwater prawn (0.04-0.50) (Imron *et al*., 2009), and nilem (0.0153-0,1392) (Mulyasari *et al*., 2010). The low value of genetic distance of flying fish *C. opisthopus* (0.003-0.025) has been revealed by isozyme technique for three populations such Tomini, Mandar, and Manado (Fahri, 2001).

The present study showed that the relatively high genetic distance between Takalar and Majene populations, reflecting the low-shared genetic drift for both populations. The other possible reason could be the physical separation of the coastal waters between the south and north of South Sulawesi continent as the geographical barrier as well as restricting the distribution of this species. The dendrogram of catfish, *M. numerus* revealed by RAPD and AFLP analysis also showed that the Serawak population was genetically different from the other populations studied in Malaysian Peninsular (Kim, 1998); while the population between Kedah and Terengganu showed a higher genetic distance compared to the other studied populations (Lim, 1998). Manezes & Parulekar (1998) pointed out that the potential genetic differentiation between populations of a species depends upon a number of variables, such as migration rate, the number of individuals within a population and natural selection at different loci.

Diagnostic Marker

The presence of species-specific marker was observed in three populations of flying fish *H. oxycephalus* for two primers. This marker is used as a diagnostic genetic marker to identify the species of flying fish. The two RAPD primers produced the diagnostic markers for flying fish at different populations. Three fragments from primer CA-01 (700 bp, 800 bp, and 1.000 bp) and four fragments (225 bp, 400 bp,

750 bp, and 850 bp) from primer P-40 were considered as species-specific markers of this present study, since they were present in all individuals from three population of flying fish.This result demonstrated the useful marker in genetic differentiation of flying fish species. Williams *et al.* (1998) also reported that a total of 15 diagnostic markers were used to identify the subspecies of largemouth bass, *Micropterus salmoides*. On the other hand, different result was shown in a study on red mullet, *Mullusbarbatus* in which the four selected primers (OPA-02, OPA-09, OPE-11, and OPE-12) failed to produce the specific marker for discriminating of different populations (Mamuris *et al*., 1998) and none of the unique fragment was found in each population of hilsa shad (Dahle *et al*., 1997). Moreover, Parenrengi (2006) also reported that the RAPD marker was successful in determining the genus-specific marker of the grouper, where five fragments (OPA02-950 bp; OPA08-950 bp; OPA16-700 bp; OPA16-550 bp; OPA17-860 bp) obviously indicated as genus-specific marker of *Epinephelus* since they were present in all three species from the same genus of studied grouper.

On the other hand, the two primers found in this present study failed to reveal the population-specific marker of flying fish. Certain fragments were present for all individuals in certain populations, but were absent in the other populations. This may be resulted from the limitation of the small sample number and the selected primers used in this present study. The other possible reason is that the individuals of each population were suspected to have the same spawning area and the larva would likely be distributed to locations of the study at the Makassar Strait area. It is also acceptable in confirming of low level genetic distance among flying fish populations under studied. However, based on its morphological analysis, Ali (2005) has reported that the flying fish *H. oxycephalus* in the Flores sea and the Makassar Strait at the stage of segregation and each is at different sub populations and different environmental controlling factors. Moreover, flying fish group of Takalar regency (part of Flores sea) with flying fish at Pare-Pare and Majene (part of the Makassar strait) have a close genetical distance, while flying fish in Pare-Pare and Majene regencies have closely related genetic. The different result of this study is predicted by the different environmental condition influencing the morphological characters of each flying fish population.

CONCLUSION

A total of 81 fragments (loci) and 50 polymorphic fragments with size ranging from 125 to 1250 bp was generated by two RAPD primers, where the high polymorphism (63.5%) was shown by Takalar population. Similarity index of individuals of intra-population was 0.60 for Takalar, 0.63 for Majene and 0.75 for Pare-Pare population. The Takalar population was genetically closer to Pare-Pare population than to Majene population. Seven fragments were identified as species-specific markers of flying fish but no population-specific markers was revealed by the two RAPD primers.

REFERENCES

Ali, S.A. 2005. *Kondisi sediaan dan keragaman populasi ikan terbang (**Hirundichthys oxycephalus** Bleeker, 1852) di Laut Flores dan Selat Makassar*. Disertasi Program Pascasarjana Universitas Hasanuddin, Makassar, 281 pp.

Asahida, T., Kabayashi, T., Saitoh, K., & Nakayama, I. 1996. Tissue preservation and total DNA extraction from fish store at ambient temperature using buffer containing high concentration of urea. *Fisheries Science*, 62(5): 727-730.

Bardakci, F. & Skibinski, D.O.F. 1994. Application of the RAPD technique in tilapia fish: species and subspecies identification. *Heredity*, 73: 17-123.

Bielawski, J.P. & Pumo, D. E. 1997. Ramdomly Amplified Polymorphic DNA (RAPD) analysis of Atlantic coast striped bass. *Heredity*, 78: 32-40.

Bowditch, B.M., Albright, D.G., Williams, J.G.K., & Braun, M.J. 1993. Use of randomly amplified polymorphic DNA markers in comparative genomic studies. *Methods in Enzymology*, 224: 294-309.

Coccone, A., Allegrucci, G., Fortunade, C., & Sbordoni, V. 1997. Genetic differentiation within the european sea bass (*Dicentrachuslabrax*) as revealed by RAPD-PCR assays. *Journal of Heridity*, 88: 316-324.

Dahle, G., Rahman, M., & Erikksen, A.G. 1997. RAPD finger printing used for discriminating among three populations of hilsa shad (*Tenualosailisha*). *Fisheries Research*, 32: 263-269.

D'Amato, M.E. & Corach, D. 1996. Genetic diversity of population of the fresh-water

shrimp, *Macrobrachium borellii* (Caridae: Palaemonidae) evaluated by RAPD analysis. *Journal of Crustacean Biology*, 16(4): 650-655.

Elo, K., Ivanoff, S., Vuorinen, J.A., & Piironen, J. 1997. Inheritance of RAPD markers and detection of interspecific hybridization with brown trout and Atlantic salmon. *Aquaculture*, 152: 55-65.

Ferguson, A., Taggart, T.B., Prodohl, P.A., McMeel, O., Thompsom, C., Stone, C., McGinnity, P., & Hynes, R.A. 1995. The application of molecular markers to the study and conservation of fish populations, with special reference to *Salmo*. *Journal of Fish Biology*, 47: 103-126.

Fahri, S. 2001. *Keragaman genetik ikan terbang,* ***Cypselurus opisthopus*** *di perairan Teluk Mandar, Teluk Manado, dan Teluk Tomini Sulawesi*. Thesis Program Pascasarjana, Institut Pertanian Bogor, Bogor, 53 pp.

Fritsch, P. & Rieseberg, L.H. 1996. The use of Random Amplified Polymorphic DNA (RAPD) in conservation genetics. In: Smith, T.B., Wayne, R.K., (Eds.) Molecular Genetic Approaches in Conservation. Oxford University Press, New York, p. 54-73.

Garcia, D.K. & Benzie, J.A.H. 1995. RAPD markers of potential in penaeid prawn (*Penaeus monodon*) breeding programs. *Aquaculture*, 130: 137-144.

Gomes, C., Dales, R.B., & Oxendford, H.A. 1998. The aplication of RAPD marked in stock discrimination of the four wing flying fish, *Hirundichthys affinis* in the central western Atlantic. *Molecular ecology*, 7: 1,029-1,039.

Gomes, C., Oxendford, H.A., & Dales, R.B. 2000. Restriction site mapping of the mitochindrial DNA of the four wing flying fish, *Hirundichthys affinis*. *DNA Sequence*, 11(3-4): 277-280.

Imron, Sunandar, D., Susilowati, R., Suprapto, R., & Khasani, I. 2009. Randomly amplified Polymorphic DNA (RAPD) fingerprinting of six Indonesian populations of giant freshwater prawn, *Macrobrachium rosenbergii*. *Indonesian Aquaculture Journal*, 4(2): 93-100.

Imron, Sunandar, D., Suprapto, R., & Listyowati, N. 2010. The application of RAPD fingerprinting to asses inbreeding levels in the cultured populations of giant freshwater prawn, *Macrobrachium rosenbergii*. *Indonesian Aquaculture Journal*, 5(1): 73-82.

Iskandariah, Kusmini, I.I., Arifin, O.Z., & Gustiana, R. 2011. Variasi genetik hasil persilangan nila BEST dengan red nifi dan nirwana menggunakan penanda RAPD. *J. Ris. Akuakultur*, 6(3): 363-371.

Jorde, L.B. 1995. Population specific genetic markers and diseases. In: Meyers (Ed.). Molecular Biology and Biotechnology: A Comprehensive Desk Reference, VCH Publisher, Inc. New York, USA, p. 724-728.

Kim, C.L. 1998. *Development of PCR-based DNA markers to identify and characterise Malaysian river catfish,* ***Mystus nemurus*** *(C&V): RAPD and AFLP*. Thesis Master of Science, Faculty of Science and Environmental Studies, Universiti Putra Malaysia, 124 pp.

Koh, T.L., Khoo, G., Fan, L.Q., & Phang, V.P.E. 1999. Genetic diversity among wild forms and cultivated varieties of discus (*Symphysodon* spp.) as revealed by Random Amplified Polymorphic DNA (RAPD) finger printing. *Aquaculture*, 173: 485-497.

Lante, S., Tenriulo, A., Parenrengi, A., Rachmansyah, & Malina, A.C. 2011. Keragaman genetik populasi ikan beronang (*Siganus guttatus*) di Selat Makassar dan Teluk Bone menggunakan metode Random Amplified Polymorphic DNA. *J. Ris. Akuakultur*, 6(2): 211-224.

Lim, F.T. 1998. Molecular polymorphisms studies of Malaysian River Catfish, ikan baung (*Mytilus nemurus*), detected using the RAPD-PCR method. Faculty of Science and Environmental Studies, Universiti Putra Malaysia, Serdang, 88 pp.

Linacero, R., Rueda, J., & Vazquez, A.M. 1998. Quantification of DNA. In: Karp, A., Isaac, P.G., Ingram, D.S. (Eds.) Molecular Tools for Screening Biodiversity: Plants and Animals. Chapman and Hall. London, Weinheim, New York, Tokyo, Melbourne, Madras, p. 18-21.

Liu, Z.J., Li, P., Argue, B.J., & Dunham, R.A. 1999. Random amplified polymorphic DNA markers: usefulness for gene mapping and analysis of genetic variation of catfish. *Aquaculture*, 174: 59-68.

Lynch, M. & Milligan, B.G. 1994. Analysis of population genetic structure with RAPD markers. *Molecular Ecology*, 3: 91-99.

Mamuris, Z., Apostolitis, A.P., Theodorou, A.J., & Triantaphyllidis, C. 1998. Application of ran-

dom amplified polymorphic DNA (RAPD) markers to evaluate intraspesific genetic variation in red mullet (*Mullusbarbatus*). *Marine Biology*, 132: 171-178.

Manezes, M.R. & Parulekar, A.H. 1998. Genetic variation in marine natural populations-measurement and utility in resource management and conservation: a review. *Indian Journal of Marine Science*, p. 267-273.

Miller, M.P. 1997. Tools for population genetic analises (TFPGA). A windows program for the analysis of allozyme and molecular population genetic data.

Miyanohara, M., Iwatsuki, Y., & Sakai, M. 1999. Analysis of the Okinawa and Miyazaki populations of the common silver-biddy, *Gerresoyena* using random amplifed polymorphic DNA (RAPD) technique. *Fisheries Science*, 65(2): 177-181.

Moria, S.B., Setiawati, K.M., Haryanti, & Wardana, I.K. 2010. Karakter genetik induk (F-0) dan turunannya (F-1) pada ikan hias laut clown (*Amphiprion percula*) menggunakan marker DNA (Random Amplified Polymorfism DNA). *J. Ris. Akuakultur*, 5(2): 183-190.

Mulyasari, Soelistyowati, D.T., Kristanto, A.H., & Kusmini, I.I. 2010. Karakteristik genetik enam populasi ikan nilem (*Osteochilus hasselti*) di Jawa Barat. *J. Ris. Akuakultur*, 5(2): 175-182.

Mwanja, W., Booton, G.C., Kaufman, L., Chandler, M., & Fuerst, P. 1996. Population and stock characterization of Lake Victoria tilapine fisher based on RAPD markers. *Aqua. Biotech. Symp. Proceeding*, p. 115-123.

Nei, M. & Li, W. 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. Proceeding of the National Academic of Science, USA, 76: 5,269-5,273.

Nessa, M.N., Mallawa, A., Najamuddin, Sadarang, A., Ali, S.A., Arifin, M.F., Alamsyah, P.M., Mardiana, & Latif, S.S. 1993. *Penelitian pengembangan potensi sumberdaya laut Selat Makassar, Laut Flores, dan Selat Makassar, Sulawesi Selatan. Lembaga Pengabdian Pada Masyarakat, Unhas, Makassar*, 235 pp.

Norazila, K.S. 2000. *Genetic polymorphisms in sea cucumbers (**Holothuridea**) using random amplified polymorphic DNA (RAPD)*. Thesis Master of Science, Faculty of Medicine and Health Science, Universiti Putra Malaysia.

Nugroho, E., Sundari, S., & Jatnika. 2011. Variasi genetik hibrida ikan gurami dianalisis dengan menggunakan marker RAPD. *J. Ris. Akuakultur*, 6(1): 1-6.

Parenrengi, A. 2006. RAPD fingerprinting of three species of grouper (*Epinephelus* spp.) from Makassar Strait, South Sulawesi, Indonesia. *Indonesian Aquaculture Journal*, 1(2): 105-119.

Parenrengi, A. & Tenriulo, A. 2008. Genetic variability and population structure of grouper (*Epinephelus suillus*) from Makassar Strait and Bone Bay, South Sulawesi, *Indonesian Aquaculture Journal*, 3(2): 77-87.

Smith, P.J., Benson, P.G., & McVeagh, S.M. 1997. Comparison of three genetic method used for stock discrimination of orange roughy, *Hoplostethusatlanticus*: Allozymes, mitochondrial DNA, and Random Amplified Polymorphic DNA. *Fishery Bulletin*, 95: 800-811.

Takagi, M. & Taniguchi, N. 1995. Random amplified polymorphic DNA (RAPD) for identification of three species of *Anguilla, A. japonica, A. australis*, and *A. bicolor*. *Fisheries Science*, 61(5): 884-885.

Williams, J.G.K., Kubelik, A.R., Livak, K.J., Rafalski, J.A., & Tingey, S.V. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic marker. *Nucleic Acids Research*, 18: 6531.