GENETIC VARIATION IN CULTURED STOCKS OF TIGER SHRIMP (*Penaeus monodon*) IN INDONESIA

Imron*), Ketut Sugama**), Komar Sumantadinata***), and Kadarwan Soewardi***)

ABSTRACT

Three stocks of tiger shrimps, *Penaeus monodon*, obtained from brackish water pond culture in Aceh (Sumatera Island), Cilacap (Java Island) and Sumbawa (West Nusatenggara) were assayed for allozyme variation at 9 enzyme loci from muscle biopsies. Three loci (*Idh*, *Gpi* and *Pgm-1*) were polymorphic in at least one of the samples. Degree of polymorphism of the Aceh and Cilacap stocks were the same (14.29), while that of the Sumbawa stock was 4.76. The average heterozygosity of the Aceh, Cilacap and Sumbawa stocks were 0.03, 0.05, and 0.017, respectively. Allele frequency differences existing among the three stocks showed that all of the three stocks did not originate from homogenous gene pools. Based on genetic distances, the Aceh and Cilacap stocks formed one group, while the Sumbawa stock formed another group. Compared with their natural population, the three cultured stocks had undergone reduction in genetic variation: 66.6% for degree of polymorphism, 24.1% for number of alleles per locus, and 26.5% for average heterozygosity, respectively.

KEYWORDS: ***genetic variation,*** *Penaeus monodon,* ***allozyme.***

INTRODUCTION

Tiger shrimp, *Penaeus monodon*, is an important commercial shrimp species in Indonesia. Although most of the annual domestic yield of penaeid shrimp comes from the harvest of wild shrimp, aquaculture industries of this species have been rapidly growing. Consequently, the need of fry as a vital production component has also been increasing.

At the beginning, the supply of fry relied on natural capture whose availability was seasonal. Fortunately, developments in the sciences and technologies, especially in reproduction, have allowed controlled mass production of fry in hatcheries (Primavera, 1978). Supply of broodstocks for mass production, however, still relies on natural population. Hatchery owners prefer to use natural broodstocks because they are larger, more fecund and produce more viable nauplii (Sugama *et al.*, 1988). Realizing the importance of natural broodstock population and as a starting point toward genetic improvement, Sugama *et al.* (1998) have studied the distribution of genetic variability and population structure of some natural penaeid shrimp populations in Indonesia.

Although up to now supply of broodstocks for fry mass production is still predominantly derived from natural sources, efforts of supplying broodstocks from rearing ponds should not be ignored. The ability of doing this is very useful in anticipating the decreasing supply of natural broodstocks caused by fishing pressure or by uncontrolled exploitation, and to keep the availability of broodstocks in appropriate quantity, quality and continuity. It is also useful in providing greater possibility to carry out genetic improvement. The weakness of cultured broodstocks especially in genetic point of view is the possibility of reduction in genetic variation due to both inbreeding and genetic drift. A multidiscipline approach is needed to avoid any loss of genetic variation.

Genetic variability data are useful as starting point for genetic improvement. In addition to genetic variability data of natural population, information on those of cultured stocks will be of great assistance in determining breeding program and genetic improvement strategies.

MATERIALS AND METHODS

Three samples of cultured stocks of tiger shrimp obtained from rearing ponds in Langsa (East Aceh), Indramayu (West Java) and Negara (Bali) were used (Figure 1). The parents of the three stocks were natural broodstocks from Aceh's, Cilacap's, and Sumbawa's waters, respectively. Sixty tiger shrimps of 125-152 mm

*) Researcher of the Research Institute for Freshwater Fisheries, Sukamandi
**) Researcher of the Research Station for Coastal Fisheries Gondol, Bali
***) Professor and Lecturer of Dept. of Aquaculture, Faculty of Fisheries, Bogor Agricultural Univ., Bogor

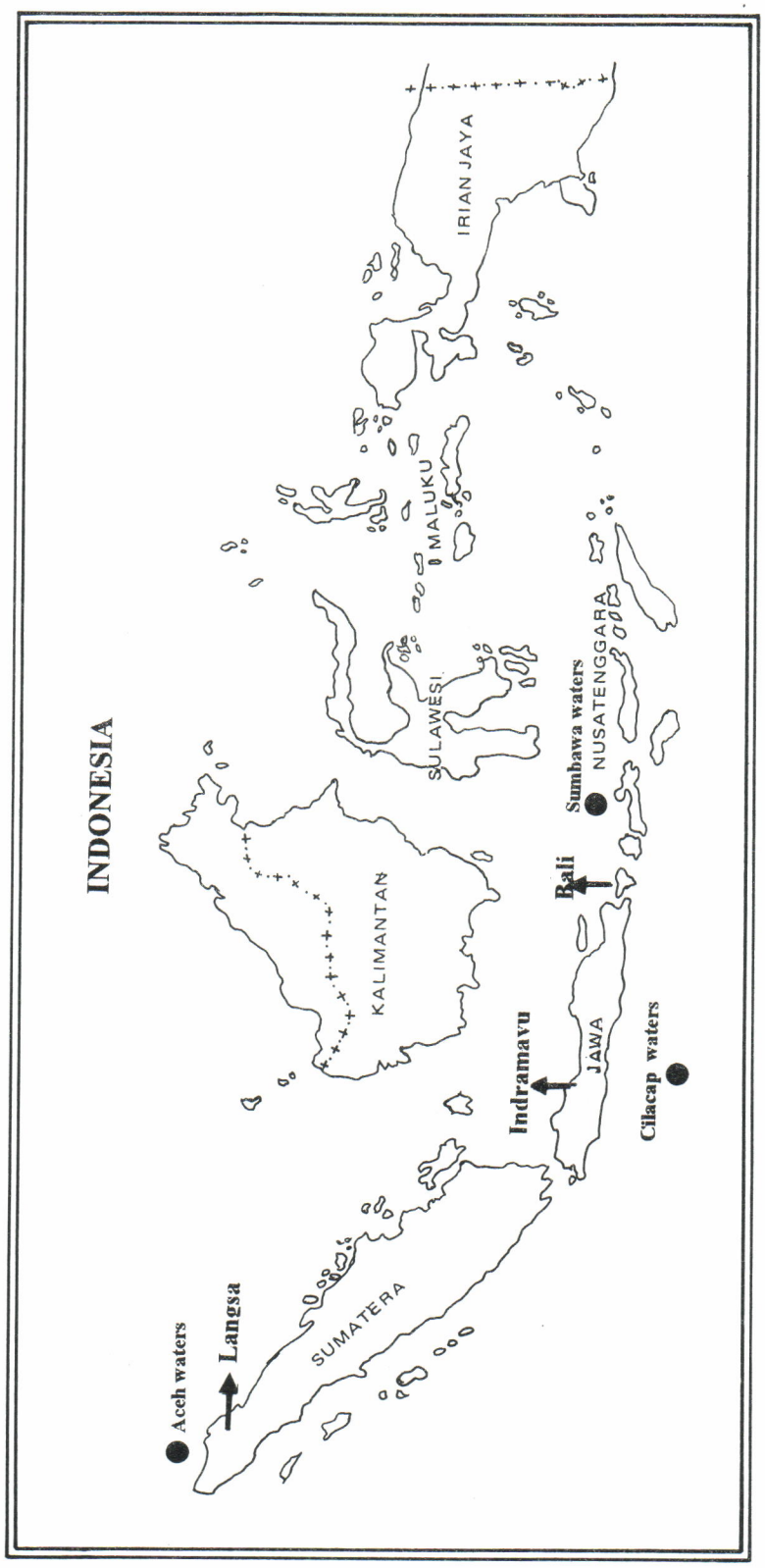

Figure 1. Geographic location of the three samples of cultured stocks of *P. monodon* (⬆) and natural waters supplying their parents (●).

in individual length and 11.5-24.1 g in individual weight represented each stock.

Horizontal starch gel electrophoresis was used following the procedures of Taniguchi & Sugama (1990). Seven enzyme systems were used to survey genetic variation. These enzymes systems were: a-glycerophosphate dehydrogenase (a-GPD, E.C. 1.1.1.8), lactate dehydrogenase (LDH, E.C. 1.1.1.27), malate dehydrogenase (MDH, E.C. 1.1.1.37), phosphoglucomutase (PGM, E.C. 5.4.2.2), glucophosphate isomerase (GPI, E.C. 5.3.1.9), isocitrate dehydrogenase (IDH, E.C. 1.1.1.42) and malic enzyme (ME, E.C. 1.1.1.40). Locus and allele nomenclature used were as described by Shaklee *et al.* (1990). Multiple loci encoding 1 enzyme separated by a hyphen (-) at the back of locus name, indicating relative migration of those loci. The fastest anodal locus was designated locus 1. Allele nomenclature was based on migration distance allele against the common allele, usually designated by allele 100.

The data obtained were used to calculate some parameters of genetic structure of population including degree of polymorphism (Leary & Booke, 1990), average heterozygosity, genetic distance (Nei, 1978), genetic similarity or dissimilarity (Nei, 1972) and genetic variation among populations (Hartl, 1980). Calculation of degree of polymorphism and average heterozygosity was based on the assumption that the number of enzymes and detectable loci were the same as obtained by Sugama *et al.* (1996). This was aimed to eliminate bias in comparing with genetic variation of natural population. Chi-square test was used to detect both the deviation of genotype frequency from Hardy-Weinberg equilibrium and heterogeneity of allele frequencies among stocks. Grouping of stocks based on similarity or dissimilarity of allele frequencies was carried out by cluster analysis and presented in dendrogram using Unweighted Pair Group Method with Arithmetic averages (UPGMA) method (Sokal & Rohlf, 1981).

RESULTS

Electrophoretic analysis results as expressed by banding patterns of zymograms showed that the seven enzymes used in this study detected 9 loci, with three of them were polymorphic at least in one of the samples, namely: Idh, Gpi, and Pgm-1. The observed phenotype, Hardy-Weinberg expectation, and allelic frequencies of the polymorphic loci in the three stocks are presented in Table 1.

Genotype frequencies of the three polymorphic loci (Table 1) showed that the genotype proportion was in Hardy-Weinberg equilibrium ($P<0.05$). There were allele frequency differences in the three stocks especially in Idh-100 and Gpi-100 alleles (Table 2). Allele frequencies of Idh-100 were significantly different between the Aceh and the Sumbawa stocks and between the Cilacap and the Sumbawa stocks. Allele frequencies of Gpi-100 were significantly different between each of paired stocks.

There were differences in degree of polymorphism (Table 3). Degree of polymorphism in the Aceh and Cilacap stocks (14.29%) were higher than that of the Sumbawa stock (4.76%). The only polymorphic locus in the Sumbawa stock was Pgm-1. The same phenomenon was also seen in the average of number of alleles per locus, where that of Sumbawa stock (1.05) was slightly lower than those of the Aceh and Cilacap stocks (1.19).

Degrees of polymorphism of all three stocks in this study were lower than those of the natural populations (Table 4). Many of the loci, which were polymorphic in natural population, became monomorphic in cultured stocks. In natural population of the Aceh and Sumbawa waters, for instance, 6 polymorphic loci were found, namely: Est, Ldh, Mdh, Idh, Pgm-1 and 6-Gpd (Sugama *et al.*, 1988) Using the same enzymes but Est, the Aceh cultured stock showed only three polymorphic loci, namely Pgm-1, Idh, and Gpi. In this study, comparison with the natural population suggested that the cultured stocks had undergone reduction in degree of polymorphism and number of alleles per locus of 67.6% and 24.1%, respectively.

Heterozygosity per locus (he) and average heterozygosity of each stock (Table 3) indicated that the Gpi contributed most to the number of heterozygotes, followed by Pgm-1 and Idh in Cilacap stock. While in the Aceh and Sumbawa stocks, the loci contributed most to the number of heterozygote consecutively in decreasing order: Pgm-1, Idh and Gpi. Average heterozygosity was highest in the Cilacap stock, followed by the Aceh and Sumbawa stocks.

Index of fixation (Fst) 0.06 among the three stocks (Table 5) indicated that genetic variability in the cultured stocks of tiger shrimp, 6%, was caused by genetic variability among the stocks, and 94% was caused by genetic variability of individuals within the stock. Genetic distance (D) between stocks ranged from 0.011 to 0.023 with average D 0.017. Genetic identity (I) ranged from 0.977 to 0.989 with average I 0.0983 (Table 3).

Table 1. Observed phenotype, Hardy-Weinberg expectation (in bracket) and allele frequency at three polymorphic loci.

Loci	Location	Phenotype						X^2	x^2 table (df, a)	N	Allele frequency		
Gpi		110/110	110/100	100/100	110/90	100/90	90/90				110	100	90
	Aceh	0	0	52	0	8	0	0.29	11.34	60	0	0.93	0.07
		(0)	(0)	(51.89)	(0)	(7.81)	(0.29)						
	Cilacap	0	10	31	0	18	1	4.01	11.34	60	0.083	0.75	0.167
		(0.41)	(7.47)	(33.75)	(1.66)	(15.03)	(1.67)						
	Sumbawa	0	0	60	0	0	0	0	11.34	60	0	1	0
		0	0	-60	0	0	0			60			
Idh		100/100	100/90	90/90								100	90
	Aceh	42	17	1				0.72	6.63	60		0.842	0.158
		(42.53)	(15.96)	(1.50)									
	Cilacap	38	22	0				3.03	6.63	60		0.817	0.183
		(40.05)	(17.94)	(2.01)									
	Sumbawa	59	1	0				0.003	6.63	60		0.992	0.008
		(59.04)	(0.94)	(0.00)									
Pgm-1		100/100	100/90	90/90								100	90
	Aceh	29	11	0				1.01	6.63	40		0.862	0.138
		(29.72)	(9.52)	(0.76)									
	Cilacap	33	27	0				5.06	6.63	60		0.775	0.225
		(36.04)	(20.92)	(3.04)									
	Sumbawa	34	26	0				4.57	6.63	60		0.783	0.217
		(36.78)	(20.39)	(2.82)									

Table 2. Heterogeneity of allele frequencies among cultured stocks of *P. monodon*.

Alleles	Chi-square		
	Aceh-Cilacap	Aceh-Sumbawa	Cilacap - Sumbawa
Idh (100)	0.265	21.206*	17.670*
Gpi(100)	17.540**	8.276**	34.286**
Pgm-1 (100)	2.388	0.024	1.995

* significantly different at 0.05 level of significance
** significantly different at 0.01 level of significance

Table 3. Summary of genetic variability of cultured tiger shrimp based on 21 enzyme loci and 1% polymorphism criterion.

	Location		
	Aceh	Cilacap	Sumbawa
Number of samples	60	60	60
Number of loci	21	21	21
Number of polymorphic	3	3	1
Proportion of polymorphic	14.29	14.29	4.76
Number of alleles per	1.19	1.19	1.05
Heterozygosity			
- per locus			
Idh (he)	0.266	0.299	0.016
Gpi (he)	0.13	0.403	0
Pgm-1 (he)	0.238	0.349	0.34
- Expected mean	0.03	0.05	0.017
- Observed mean	0.04	0.06	0.007
- Ho/He	1.33	1.2	0.41
	Aceh-Cilacap	*Cilacap -Sumbawa*	*Aceh - Sumbawa*
Genetic Identity (I)	0.989	0.977	0.983
Average genetic identity	0.983 ± 0.005	0.983 ± 0.005	0.983 ± 0.005
Genetic distance (D)	0.011	0.023	0.017
Average genetic distance	0.017 ± 0.005		

Table 4. Comparison of average genetic variability between natural population and cultured stock of *P. monodon*. Both natural population and cultured stock were represented by Aceh and Sumbawa samples. Natural population data were from Sugama *et al.* (1996).

	Natural population (2 lots)	Cultured stock (2 lots)	Reduction (%)
Number of samples	100	60	
Number of loci	21	21	
Number of polymorphic loci	6 ± 0	2 ± 1	66.6
Proportion of polymorphic loci (%)	28.5 ± 0.66	9.5 ± 0.048	66.6
Number of alleles per locus	1.475 ± 0.475	1.120 ± 0.070	24.1
Heterozygosity	0.037 ± 0.009	0.023 ± 0.006	26.5
F_{st}	0.009	0.005	

Table 5. Values of F_{st} based on alleles frequency in the three cultured stocks of *P. monodon*.

Loci	Alleles	F_{st}
Idh	100	0.058
	90	0.058
Gpi	110	0.116
	100	0.064
	90	0.1
Pgm-1	100	0.01
	90	0.01
Average		0.060 ± 0.037

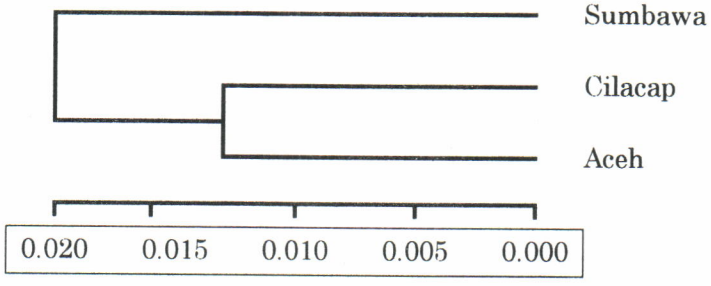

Figure 2. Dendrogram showing the genetic relationship among three stocks of cultured tiger shrimp using Nei genetic distance (1978) as the metric and UPGMA clustering method.

These values of genetic variability among the cultured stocks were slightly higher than those of the natural population (Table 4). Dendrogram of genetic distance between stocks (Figure 2) indicated that there were genetic differentiations in the three stocks. The Aceh stock and Cilacap stock were relatively close compared to the Sumbawa stock.

DISCUSSION

In general, the low genetic variability exhibited by the level of polymorphism, number of alleles per locus and average heterozygosity in the cultured stocks suggested that cultured tiger shrimp have undergone a reduction in genetic variability. Factors that could cause this reduction are the founder effect that can take place through broodstock selection, inbreeding or a bottleneck effect. Since all of the hatcheries used natural broodstocks without selection activities, then it is unlikely that selection process and inbreeding have caused the reduction in genetic variability. Rather, random genetic drift or bottleneck effect caused by a small founding population in the hatcheries is probably the main factor causing the reduction of genetic variability in the cultured stocks. According to Allendorf & Phelps (1980) there are three major changes in genetic variability of population caused by random genetic drift: reduction in polymorphic loci, number of alleles per locus and average heterozygosity. Those changes were clearly seen in this study (Table 4).

The number of broodstocks usually used for fry production in hatcheries is based on the production target. The larger the production target, the larger the number of broodstocks used. In terms of genetic variability, it is clear that the small number of broodstock used in the hatcheries resulted in the high probability of founder effect random genetic drift, because the number of broodstocks taken as spawners in hatcheries did not fully represent the gene pool of the natural population. The smaller the number of founder population, the larger the probability of genetic drift. These were observed in all of the cultured

stocks studied. The loss of some polymorphic loci in the cultured stocks was probably an indicator of the occurrence of random genetic drift associated with the unrepresentative samples. The loss in genetic variation which might be due to small number of effective parents was also already observed in hatchery stock of brown trout (Ryman & Stahl, 1980)

In addition to the small number of broodstocks, it was estimated that fry production management at hatcheries also contributed to the decline of genetic variation in cultured broodstocks. Fry production management practiced by a hatchery in Aceh as will be described below for example, perhaps can explain the low genetic variation in cultured stocks. A hatchery owner uses about forty broodstocks to obtain fry production target of 4 million fry per cycle. Each broodstock is placed in 5-ton concrete tanks which functioned as egg releasing tanks, larva rearing tanks as well as post larva rearing tanks. Generally all of the developmental stages from the hatching to the ready to stock post larva take place in the same tank and there has no mixing process with offspring from another broodstocks during that period. Because of the high fecundity, usually one hundred thousands of ready to stock fry can be produced from each broodstock. The fry harvested from that post larva rearing tanks usually then are stocked in a growing pond without any mixing with fry from another broodstocks, so the genetic variation of shrimp at that pond comes from just one pair of broodstocks. As a result, although the total numbers of broodstocks used at the hatchery are of significant number, the actual parent of the sample shrimps used in this study might be just one or two broodstocks. Fry production management such as this, of course, can considerably reduce the genetic variation in cultured stocks. Ryman & Utter (1987) had discussed widely the management practices of hatchery stocks resulting in the reduction of genetic variation.

Reduction of genetic variability in the cultured stocks in this study was similar to that reported by Taniguchi *et al.* (1983) for the hatchery stock of black sea bream, Stahl (1983) for the hatchery stock of Atlantic salmon, and Sunden & Davis (1991) for the cultured stock of tiger shrimp.

Genetic variability in the cultured stocks, to some extent reflected genetic variability of the natural population from which the parents of the cultured stocks had been taken. The causes of genetic differentiation among stocks, therefore, can be explained to some extent by factors causing genetic variability differences in the natural population. The low degree of polymorphism in Sumbawa stock, for instance, may be traced to the genetic variability of the natural population of broodstock in Sumbawa waters. The natural population of shrimp in Sumbawa waters, from which the broodstocks of Sumbawa hatcheries were taken, had frequencies of homozygote genotype at major allele (allele-100) which were higher in all of polymorphic loci detected compared to those of the natural population of Aceh waters (Sugama *et al.*, 1996). As a result, the probability of occurrence for individuals of homozygote genotype at any locus in Sumbawa waters shrimp chosen as broodstock in hatchery were higher than those of Aceh waters.

Differences in allele frequency existing among the three stocks indicated that they did not come from homogenous gene pools. This might be caused by geographic barrier in reproduction, i.e. individuals tend to mate with those from the same geographic region. In addition, allele frequency variation was also related to adaptive patterns to the environment. A study carried out by Powers & Place (1978) reported the presence of an allele cline from the north to the south in *Fundulud heteroclitus* population. This phenomenon seems to be associated with an environmental gradient. They found the Ldh-B allele in *Fundulus heteroclitus* which had two primer alleles, varied in frequency according to the distribution of that species. The highest frequency of Ldh-B^b allele was found in the north Atlantic coast and the lowest was in the south Atlantic. This high frequency of allele seemed to be associated with temperature. This allele more often appeared in warm region and rarely in cold region. This prediction was supported by Yardley *et al.* (1974) who found that gene frequency varied between lentic and lotic environments in mosquito fish, *Gambusia affinis* and largemouth bass, *Micropterus salmoides*. Whether the previous results can explain the phenomenon emerging in this research, however, need further assessment.

Closeness in genetic distance of Aceh and Cilacap cultured stocks might be caused by genetic introgression. Many hatcheries in Java use broodstocks from Aceh waters. Likewise in aquaculture industries, many farmers in Java prefer to use tiger shrimp fry from Aceh broodstocks. As a result, there is a high probability that offspring of Aceh broodstocks, through various ways, have entered Java Island waters and developed to broodstocks, then are chosen as broodstock for hatcheries in Java.

Difference in average heterozygosity (H) might be caused by difference in adaptive patterns to different environments. According to Nelson & Hadgecock (1980), maximum heterozygosity at Gpi locus positively correlated with the waters productivity and trophic instability. Heterozygosity at the Pgm locus was positively correlated with opportunism and negatively with trophic levels. Average hetorozigosity positively correlated with productivity and trophic stability. The values of heterozygosity ranging from 0.017 to 0.05 were similar or slightly higher than previous results, both in natural population and cultured stocks. Heterozygosity in population of *P. monodon* of Mexico, Panama and Equador waters were 0.017, 0.017 and 0.021, respectively, while that of the cultured stocks in Equador was 0.011 (Sunden & Davis, 1991). Based on Nelson & Hadgecock (1980) on the correlation between heterozygosity and waters productivity, it may be predicted that there were differences in waters productivity among localities in Indonesian waters as well as other countries. Whether this conclusion is correct, however, still need more study.

The average genetic distance (D) of 0.017 suggested that the three stocks were local populations of the same species (Avise, 1978). Dendrogram of cultured stocks constructed based on genetic distance showed the same pattern as that from natural population (Sugama *et al.*, 1988).

CONCLUSION

Based on some indicators of biochemical variability, the Cilacap stock had the highest variability followed by the Aceh and Sumbawa stocks. The three stocks were the local populations of the same species. Genetic variability of the cultured tiger shrimp stock had undergone reduction in degree of polymorphism, number of alleles per locus and average heterozygosity compared with the natural population. It is recommended to increase the number of broodstocks and apply appropriate fry production management strategies to minimize the decline of genetic variability in cultured stocks.

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