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GENETIC DIVERSITY IN TIGER PRAWN (*Penaeus monodon*) FROM TWO FARMS ON THE WEST COAST OF SABAH, MALAYSIA

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ABSTRACT. Pond-raised tiger prawn (*Penaeus monodon*) samples collected from two aquaculture farms on the west coast of Sabah were subjected to starch gel electrophoresis to reveal genetic variability. Seven enzymes were analyzed: alcohol dehydrogenase, phosphoglucomutase, isocitrate dehydrogenase, phosphogluconate dehydrogenase, glucose-6-phosphate dehydrogenase and glucose-6-phosphate isomerase. The study managed to score nine loci out of which four were polymorphic in specimens from both the farms. The polymorphic loci were *GPI, IDH, PGDH* and *PGM*. The remaining monomorphic loci were *ADH-1, ADH-2, G3PDH-1, G3PDH-2* and *G6PDH*. The proportion of polymorphic loci was relatively high (44.4%). Mean expected heterozygosity was 0.219±0.086(S.E) and 0.207±0.084 (S.E) in KO-NELAYAN tiger prawn farm and Likas farm, respectively. The number of allele per locus was low: 1.6±0.2 (S.E) in Likas farm population and 1.4±0.2 (S.E) in KO-NELAYAN farm population. Chi-square analysis showed that the data significantly deviated from expected Hardy-Weinberg value. However, the deviation has been shown to increase the number of heterozygote individuals. This indicated that the analyzed tiger prawn samples were a product of wild population, and there was no inbreeding depression. Suggestions have been outlined in this paper on steps to be taken to maintain the genetic variability if the tiger prawn broodstock raised in these farms is to be used for high quality seed production.

KEYWORDS: Allozyme electrophoresis, fishing pressure, genetic variation, pond, tiger prawn.

INTRODUCTION

Sabah is the main producer of pond-raised tiger prawn in Malaysia (NACA & DOF, 1996). Major part of the production is consumed locally, while the rest is exported. Due to ever increasing demand and considerably high levels of exploitation, the wild population of

tiger prawn is declining (NACA & DOF, 1996). Therefore, aquaculture production of tiger prawn in Sabah is important to bridge the gap between supply and demand. The tiger prawn is cultured in earthen ponds generally constructed in mangrove areas. Gravid females caught from sea and held in captivity for breeding provide the seed for stocking in the ponds. Aquaculture of tiger prawn in Sabah has been progressing until 1997. Declined in 1998 and intensified in subsequent year (DOF, 2001). This setback was linked to poor egg quality, excessive larval mortality, and outbreak of diseases (DOF, 2001). Genetic factor, if any, associated with seed quality or vulnerability to infections diseases was not investigated. The present study reports results of our investigations on genetic composition of tiger prawn (*Penaeus monodon*) from two commercial hatcheries in Sabah.

MATERIALS AND METHODS

Fifty-eight samples of pond-raised *P. monodon* were collected from two farms on the west coast of Sabah to reveal genetic variability. One of the farms is located in Likas and is owned by a private company. The other one is in Tuaran and belongs to KO-NELAYAN (Koperasi Kemajuan Perikanan dan Nelayan). Prawn samples were brought to the laboratory alive. Once in the laboratory, the samples were dissected and abdominal muscle tissue was removed for allozyme assays. Tissues were homogenized using Tris-HCl (pH 8.0) as described by Aebersold *et al.* (1987). Samples were then subjected to starch gel electrophoresis following the procedure described by Ballment *et al.* (1993). Starch gel concentration used in this study was slightly lower (11.5%) than that suggested (12%) in the standard manual by Aebersold *et al.* (1987). The gel buffer used was TBCL (Aebersold *et al.* 1987). Electrophoresis was carried out at 250 V, 70mA for 5 hours. A temperature of 4°C was maintained. Bromophenol blue solution (5%) was used as the indicator. Starch gel was then sliced into several pieces and stained for different allozymes following the standard method described by Aebersold *et al.* (1987). However, uppermost layer of the starch gel was removed as it normally produces inconsistent allozyme banding patterns (Ransangan *et al.*, 1999). Genotype frequencies were manually calculated and subsequently analyzed using Biosys-1 (Swofford and Selander, 1989). Test for deviation from Hardy-Weinberg equilibrium was performed using Chi-square analysis. Loci were considered polymorphic if the frequency of the most common allele did not exceed 0.95. Genetic distance measured in terms of gene frequencies averaged over all loci in different populations was based on Nei's unbiased minimum distance (Nei, 1978).

RESULTS

Of the seven enzymes examined in tiger prawn specimens from both the ponds, 9 loci were scored. Four of them (*GPI, IDH, PGDH* and *PGM*) were polymorphic while remaining five (*ADH-1, ADH-2, G3PDH-1, G3PDH-2* and *G6PDH*) were monomorphic.

The two populations have similar levels (44.4%) of polymorphic loci. The mean observed heterozygosity (H_o) in prawn from Likas and KO-NELAYAN ponds was 0.219±0.086 and 0.207±0.084, respectively. The number of alleles per locus was 1.6±0.2 in Likas pond population and 1.4±0.2 in KO-NELAYAN pond population. The polymorphic loci exhibited a significant deviation from Hardy-Weinberg equilibrium (P<0.001). Heterozygosity at individual locus was also recorded for all the polymorphic loci in both populations. The level of observed heterozygosity for *GPI* and *IDH* allozymes in Likas pond population was 0.508 and 0.310, respectively whereas for allozymes *PGM* and *PGDH* it was 0.534 and 0.508, respectively. Observed heterozygosity in KO-NELAYAN pond stock was 0.520 (*GPI*), 0.493 (*IDH*), 0.462 (*PGM*) and 0.498 (*PGDH*).

The four polymorphic loci in KO-NELAYAN pond population (*PGM, IDH, PGDH* and *GPI*) significantly deviated from Hardy-Weinberg equilibrium (P<0.001) but the deviation seemed to increase the number of heterozygote individuals. Similar trend was also observed for these polymorphic loci in Likas pond population. Based on Nei's (1978) genetic distance analysis, there was no genetic gradient in the populations. This view is strengthened by the fact that the unbiased minimum genetic distance is low (0.008) and similarity as high as 99%. Variations in terms of Nei distance appeared to be contributed by two loci (*PGM* and *IDH*). The mean F_{ST} for the analyzed populations was 0.027. The allele frequencies (Table 1) of the four polymorphic loci found in the two populations studied was consistent except for an allele (*PGM-1 [80]*) in Likas pond population that was not found in KO-NELAYAN pond specimens and its allele frequency was low (0.031).

DISCUSSION

Observed heterozygosities (0.207-0.219) in the two populations of pond-raised tiger prawn are in the range of values reported for wild population of *P. monodon* obtained through microsatellite studies (0.933-0.425) and mitochondrial DNA (mtDNA) analyses (0.537 – 0.682)(Benzie, 2000). The values were, however, higher than those (0.000-0.103) reported through allozyme studies (Benzie, 2000). It is interesting to note that the mean observed heterozygosities were higher than the value (0.016) obtained by Ko *et al.* (1983) for hatchery stock of *P. monodon*. The four polymorphic loci scored in this study were the same as reported by Ballment *et al.* (1993) for Australian wild populations of *P. monodon*.

The proportion of polymorphic loci was a little higher (0.444) but within the range of 1.000 –0.081 obtained on wild population of *P. monodon* (Benzie, 2000). There was slightly different trend noticed in terms of the proportion of polymorphic loci (0.069) in cultured *P. monodon* (Ko *et al.*, 1983). The mean number of alleles per locus (1.4-1.6) was also consistent with other studies on wild *P. monodon* (1.4 –3.0). There is no information on the mean number of allele per locus available for cultured *P. monodon* (Benzie, 2000).

Table 1. Allele frequencies and genetic variability of tiger prawn specimens from Likas and KO-NELAYAN ponds

Locus	Populations			
	KO-NELAYAN Pond	Heterozygosity	Likas Pond	Heterozygosity
GPI				
(N)	26		32	
119	0.5	0.51	0.5	0.508
100	0.5		0.5	
IDH				
(N)	26		32	
157	0.423	0.498	0.188	0.31
100	0.577		0.813	
PGM				
(N)	26		32	
100	0.654	0.462	0.531	0.534
90	0.346		0.438	
80			0.031	
PGDH				
(N)	26		32	
120	0.577	0.498	0.5	0.508
100	0.423		0.5	
Mean		0.219±0.086		0.207±0.084
No. alleles per locus		1.4±0.2		1.6±0.2
Polymorphic loci		44.40%		44.40%

N=Number of individuals analyzed

The genetic distance of the two populations of *P. monodon* analyzed in this study was 0.008. This seems to be in the range of values obtained through other allozyme studies (0.000-0.015) and Random Amplified Polymorphic DNAs (0.032-0.070) (Benzie, 2000). The F_{st} of the *P. monodon* was 0.027. This value too was in agreement with results (0.007-0.031) of other studies on *P. monodon* (Benzie *et al.*, 1992; Sodsuk *et al.*1992; Forbes *et al.*, 1999). Evidently, there is no significant genetic difference within as well as between populations. Genetic structuring among the examined populations is unlikely.

Based on the levels of heterozygosity, proportion of polymorphic loci and mean number of alleles per locus, it seems *P. monodon* samples from the hatcheries are a product of wild population and have not suffered from inbreeding depression. Although, the prawn

populations in Sabah have considerable amount of genetic diversity, measures should be taken to ensure that it remains preserved through generations. Greatest threat will be the fishing pressure. This species is heavily exploited for food and for procurement of spawners. Higher price of tiger prawn intensifies the fishing activities (DOF, 2001). That overfishing can alter the genetic diversity is evident from the work of Smith *et al.* (1991). Considerable amount of literature has been published on the effect of fishing pressure on genetic diversity of marine vertebrates like the orange roughy in three fishing grounds in New Zealand. The heterozygosity of individual locus was studied in 1982 and repeated in 1988. Two loci (*IDH-1* and *GPI-2*) were scored, and over the years heterozygosity appeared to decline. The heterozygosity of *IDH-1* and *GPI-2* in 1982 was 0.25 and 0.11, respectively but reduced to 0.04 and 0.00 in 1988 as the fishing pressure mounted (Smith, *et al.*, 1991). Uncontrolled fishing activity removes the older individuals from the fishing grounds, leaving the younger ones that have reduced proportion of heterozygotes (Smith *et al.* 1991). This observation is supported by considerable amount of literature on the positive correlation between heterozygosity and growth rate or size (Mitton and Grant, 1984; Zouros, 1987; Zouros and Foltz, 1987). Another example of negative impact of heavy exploitation on genetic diversity was the shift of maturity age of Atlantic cod from 8 to 10 years in 1930 to 6 year in 1970 (Barisov, 1979). Similar observation was also reported for wild population of haddock (*Melanogrammus aeglefinus*) where in early 1970s the maturity age of female and male haddock increased from 3.3-4.6 to 4.3-5.9 years (Templeman and Bishop, 1979). However, the pattern was changed in 1983 when female matured at the age of 4.4 to 3.0 years while male matured at the age of 3.9 to 2.7 years (Beacham, 1983).

Use of limited number of parental stock in captive breeding leads to genetic erosion and development of abnormalies. The study conducted by Benzie (2000) on *P. monodon* reported that the observed mean heterozygosity for wild population was 0.026 but reduced to 0.016 in cultured population. Similar observations were also reported for *P. japonicus* where observed mean heterozygosity for wild population was 0.047 but reduced to 0.043 in cultured population (Sbordoni *et al.*, 1986). Captive breeding of *P. stylirostris* reduced the level of heterozygosity from 0.060 in wild to 0.041 in hatchery stock (Ko *et al.*, 1983). Another study conducted by Sbordoni *et al.* (1986) through seven generations of *P. japonicus* reported that level of heterozygosity dramatically reduced from 0.102 in the first captive generation to 0.036 in the seventh generation. Similar trend was also reported for some salmonids species such as Atlantic salmon in Finland where mean observed heterozygosity in wild population was 0.056 but 0.041 in its hatchery counterparts (Koljonen, 1989).

Reduction in allele frequencies of some cultured marine species has been reported in the work of Gaffney *et al.* (1992) and Ryman and Ståhl (1980). The PGM [80] with the frequency of 0.031 in Likas pond population can be considered vulnerable in case founder effect takes effect in hatchery propagation.

CONCLUSION

Hatchery stock of *P. monodon* in Sabah has not suffered genetic erosion. To preserve its genetic diversity, seed production should be based on an adequate number of brood specimens. Since wild stock is used to generate seed, any genetic change in natural population due to overfishing will invariably produce genetic modification in the progeny. Such a change will not be related to contemporary hatchery practices in Sabah but to fishing pressure on the natural stocks.

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