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# Allozyme variability analysis in striped catfish (*Pangasianodon hypophthalmus*, Sauvage, 1878) of some hatchery stocks in Bangladesh

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**KEYWORDS**

Heterozygosity, polymorphism, Allozyme electrophoresis, Genetic conservation

**A B S T R A C T**

Striped catfish (*Pangasianodon hypophthalmus*, locally called *Thai pangas*) is currently one of the largest intensively cultured fish species in Bangladesh and its fry production largely depends on commercial private hatcheries. In order to assess the genetic structure of hatchery stocks of *Thai pangas*, four hatchery samples, namely *Belal*, *Bhai-Bhai*, *Bhai-Bon* and *Jahangir* from Bogra region were collected for allozyme analysis. Five enzymes EST, GPI, LDH, MDH and PGM were studied in CA6.1 buffer system and three enzymes ADH, G3PDH and IDHP were studied in TG-1, using horizontal starch gel electrophoresis. The enzymes were encoded by 11 gene loci: Adh-1*, Est-1*, G3pdh-1*, Gpi-1*, Gpi-2*, Idhp-1*, Ldh-1*, Ldh-2*, Mdh-1*, Mdh-2* and Pgm*. Within these eleven screened loci, Est-1*, G3pdh-1*, Gpi-2* and Pgm* showed heterozygosity in all the populations studied. The mean proportion of polymorphic loci per population was 36.36% for all the populations studied. The mean proportion of heterozygous loci per individual was found to be 13.33% for all populations. The highest variability measured by the mean number of alleles per locus was 1.545 in *Bhai-Bon* population. Among the four populations the heterozygosity ratio ($H_o/H_e$) was the highest (0.636) and the 1- $H_o/H_e$ value was the lowest (0.364) in *Belal* population. The co-efficient of gene differentiation ($G_{ST}$) of four hatchery populations of *Thai pangas* was calculated 0.614 suggesting higher genetic differentiation among populations. The UPGMA dendogram constructed from Nei's (1972) genetic distance revealed that *Belal* and *Bhai-Bon* populations having lowest genetic distance (D=0.005) constructed a cluster and they were differentiated from the other cluster formed by *Bhai-Bhai* and *Jahangir* population was D=0.01. The studied populations were geographically close to each other but the *Belal* population showed greater variation. There is a clear indication that the exchange of broodstock or using broods from the same sources might be the reason for the low genetic distance in the populations of the two clusters (D=0.005 and 0.01). Based on Nei's genetic distance criteria, the four studied hatchery populations of *Thai pangas* may be categorized as local race or population.

## Introduction

Striped catfish (*Pangasianodon hypophthalmus*, locally called *Thai pangas*) is one of the most widely cultured fish species of Bangladesh due to its faster

growth, disease resistance and its adaptability to high density culture system (Sarker, 2000). The species was introduced in Bangladesh in 1990 from Thailand by the Ministry of Fisheries and Livestock (MoFL). It has received considerable commercial importance in major freshwater aquaculture areas including Mymensingh, Jamalpur, Sherpur, Narshingdi, Jessore, Bogra, Kushtia, Naogaon, and many other parts of the country and currently accounts for approximately 29% of total aquaculture production together with tilapia (Belton et al. 2011b). This rapid expansion in aquaculture occurred after successful induced breeding of this species was initiated in 1993.

Currently large-scale fry and fingerling production of *Thai pangas* is being carried out in more than 400 private and 20 government hatcheries in Bangladesh (DOF, 2011). Up on its introduction in the country, broods were brought from Thailand and later from Vietnam and the hatchery owners collected them and used for producing hatchlings and fry. It is assumed that recruited broods are frequently bred and are not replaced at regular interval for production of fry. The genetic deformities are obvious if family selection and pedigree mating are ignored.

Alam et al. (2004) conducted some genetic analysis study of *Thai pangas* hatchery populations in Mymensingh region and reported that four hatchery populations in that region were not geographically isolated and one hatchery population has lost its genetic variability. Researchers have proposed that understanding the genetic variation within domestic catfish populations is a main requirement for maximizing the selective breeding of the respective species (Perales-Flores et al. 2004). It is, therefore, essential to determine the genetic status of different *Thai pangas* populations being used in hatcheries in other parts of the country including Bogra, Jessore, Gazipur districts.

Allozyme electrophoresis is a molecular marker technology used as an effective tool for fish population studies and fishery management (Utter, 1991). With the allozyme electrophoretic separation technique, the protein product of a given gene is assayed and genetic polymorphism is studied by comparing protein mobility in an electrical field. Electrophoretic data then could be used to define population structure or to estimate inter-population gene flow through the analysis of genotype frequencies at multiple and/or independent loci. The present research was undertaken to observe the genetic status of population of *Thai pangas* to indicate if any genetic variability happened among hatchery populations of Bangladesh.

## Materials and methods

### Fish samples

The fry of *Thai pangas, P. hypophthalmus* were collected from four different hatcheries i.e., Balal Uddin Hatchery, Bhai Bhai fish farm, Bhai Bhai fish seed plant and Jahangir fish farm, Bogra, Bangladesh. Details of sampling localities, number of samples and collection date are given in Table 1. Thirty individuals from each population were caught randomly to collect trunk muscle tissues for allozyme work. The collected muscle tissues were put in small, previously marked air-tight plastic bags and stored in a freezer (-18°C) immediately. This storage system continued until completion of the electrophoretic analysis.

**Table.1** Population of *Thai pangas* used in present study

| Population number | Population | No. of individuals collected |
|---|---|---|
| 1 | Belal Uddin Hatchery (BaanerPukur, Kahalu, Bogra) | 200 |
| 2 | Bhai Bhai fish farm (Laxmipur, Kahalu, Bogra) | 200 |
| 3 | Bhai Bhai fish seed plant (BaanerPukur, Kahalu, Bogra) | 200 |
| 4 | Jahangir fish farm (Shaghata, Kahalu, Bogra) | 200 |

### Allozyme electrophoresis

In the experiment, horizontal starch gel electrophoresis (Shaw and Prasad, 1970) method was used for allozyme work. The enzymes analyzed, E.C numbers, abbreviation of enzymes, enzyme patterns and tissue type for horizontal starch-gel electrophoresis are shown in Table 2. After completion of electrophoresis, the gel was sliced horizontally into five or more sections, depending on the thickness of the gel. Loci were numbered consecutively from the anodal to cathodal side. Thus, the most anodal locus was designed "1". Gene nomenclature was followed by Shaklee et al. (1990). The electrophoretic bands corresponding to multiple alleles at each locus were alphabetically named as *a, *b, *c…in the order of detection.

### Genetic analysis

Allele frequencies were calculated directly from the observed genotypes. The distribution of the observed genotypes was compared with the expected ones, calculated from the Hardy-Weinberg equilibrium using a chi-square ($\chi 2$) test. When the most common (major) allele existed in a frequency less than or equal to 0.95 at a given locus, the locus was regarded as polymorphic. The mean proportions of heterozygous loci per individual, mean proportions of polymorphic loci per population and average number of alleles per population were calculated so as to show the extent of genetic variability for each population (Lewontin and Hubby, 1966). Expected (*He*) and observed heterozygosity (*Ho*) were also calculated (Nei, 1972). Coefficient of genetic variation ($G_{ST}$) was also calculated in order to estimate diversity between sample lots. Genetic distance values (*D*) (Nei, 1972) were calculated from allelic frequencies for all possible pairs of sample lots. The analysis of allozyme data were performed using POPGENE, version 1.32 (Yeh et al. 1999) and G-Stat, version 3.1 (Siegismund, 1995) and TREEVIEW (Page, 2000) package computer program. Based on the *D*-values, dendogram and radial tree were made by the unweighted pair group method using arithmetic average (UPGMA) method (Nei, 1987).

## Result and Discussion

### Alleles, genotypes and allele frequencies

The electrophoretic patterns of muscle tissue showed that the enzymes were controlled by the genes at 11 presumptive loci where three genotypes (*aa, *ab and *bb) were found for the two loci (Gpi-2* and G3pdh-1*), four genotypes (*aa, *ab, *bb, *bc) for Est-1*, five genotypes (*aa, *ab, *bb, *bc and *cc) for Pgm* and only one genotype (*aa) was observed for Gpi-1*, Ldh-1*, Ldh-2*, Mdh-1*, Mdh-2*.

Allele frequencies were calculated directly from observed genotypes at eleven loci in 120 samples from four populations (Table 3). Among the eleven loci, all the population showed four polymorphic loci (Est-1*, Gpi-2*, G3pdh-1* and Pgm*).

The dimeric enzyme alcohol dehydrogenase (ADH) exhibited three banding patterns consisting of one homodimer and two heterodimers, presumably controlled by 2 loci, Adh-1* and Adh-2*. However, Adh-2* was not readable due to complex banding pattern and low reproducibility. Monomeric esterase (EST) exhibited one homodimer and two heterodimers, presumably controlled by at least 2 loci. Three alleles (*a, *b and *c) were deteccted in anodal Est-1*. Allele *a was dominant in Bhai Bhai and Jahangir populations and the frequency ranged fro 0.78 to 0.87. On the other hand, allele *b was dominant in Belal and Bhai-Bon populations and the frequency ranged from 0.53 to 0.62 (Table 3). The dimeric enzyme glycerol-3-phosphate dehydrogenase (G3PDH) exhibited three banding patterns.

The G3pdh-1* was heterozygous by two alleles *a and *b, where *b was dominant in all the populations and the frequency ranged from 0.80 to 0.93. Glucose-6-phosphate isomerase (GPI) was supposed to be controlled by two loci, Gpi-1* and Gpi-2*. Gpi-1* was monophorphic with frequency *a=1.00 while Gpi-2* locus was heterozygous with two alleles (*a and *b). Allele *a was dominant in all the populations and the frequency ranged from 0.58 to 0.87. Dimeric isocitrate dehydrogenase (IDHP) exhibited monomorpic locus Idhp-1* and the tetrameric lactate dehydrogenase (LDH) showed two loci Ldh-1* and Ldh-2*, monomorphic in all the populations. The malate dehydrogenase (MDH) was controlled by at least two different loci Mdh-1* and Mdh-2*.

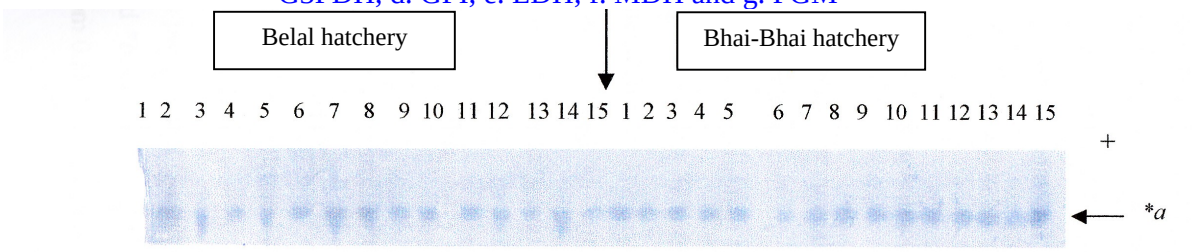
The phosphoglucomutase (PGM) was monomeric enzyme controlled by the single locus Pgm*. Three alleles *a, *b and *c were detected. Allele *a was dominant in all the populations except in Bhai-Bhai, and the frequency ranged from 0.52-0.63. In the Bhai-Bhai population, allele *b was dominant with frequency of *b=0.48. Allele *c was rare in the Bhai-Bhai, Bhai-Bon and Jahangir populations and the frequency ranged from 0.03-0.15. Allele *c was not observed in Belal population.

The chi-square ($\chi 2$) test was made in all the cases of polymorphic loci between observed and expected genotypes based on Hardy-Weinberg equilibrium. The Belal population showed significant variation in allele frequencies in four loci Est-1*, G3pdh-1*, Gpi-2* and pgm*, Bhai Bhai populations in three loci Est-1*, Gpi-2* and pgm* and Jahangir population in three loci G3pdh-1*, Gpi-2* and Pgm*. On the other hand, Bhai-Bon population showed significant variation in allele frequencies in two loci Gpi-2* and Pgm* (Table 4).

## Genetic variability

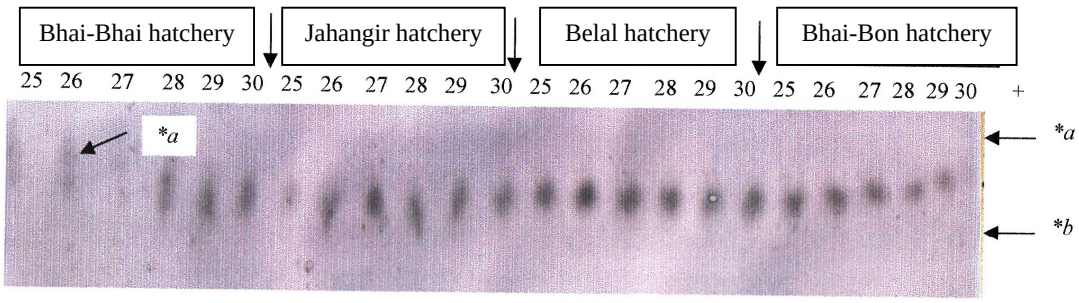
The mean proportion of polymorphic loci per population was 36.36% in all population. The mean proportion of heterozygous loci per individual for all populations was 13.33%. The average number of alleles per locus was 1.455 ± 0.07 for the populations and ranged from 1.364 (Belal population) to 1.545 (Bhai-Bon population). The average observed heterozygosity ($H_o$) for the populations was 0.072 ± 0.028 and ranged from 0.039 (Jahangir population) to 0.103 (Belal population).

**Fig.1** Electrophoregram of different locus in *Thai pangas*, a. with enzyme ADH, b. EST, c. G3PDH, d. GPI, e. LDH, f. MDH and g. PGM



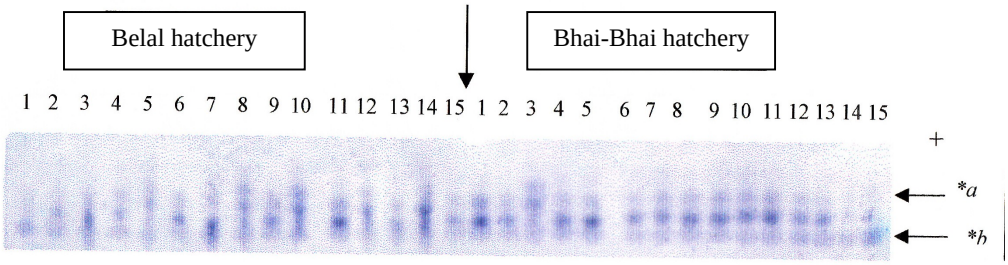

*Adh-1*:* *aa* aa* aa* aa* aa* aa* aa* aa* aa* aa* aa* aa* aa* aa* aa* aa* aa* aa* aa* aa* aa* aa* aa* aa* aa* aa*aa* aa* aa* aa*aa* aa*aa**

**Fig 1 (a)**



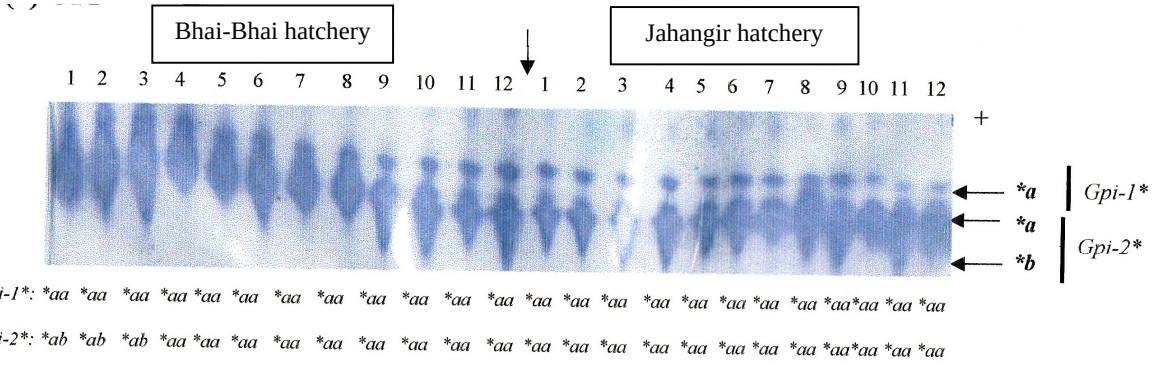

*Est-1*:* *ab *ab *aa *ab *bb *bb *bb *bb *bb *bb *bb *bb *bb *bb *bb *bb *bb *bb *bb *bb *bb *bb *bb *bb *bb *bb *bb *bb *bb

**Fig 1 (b)**




*G3pdh-1*:* *bb* bb* bb*bb*aa *bb*bb *ab*bb *ab*bb*aa *bb *aa*bb *bb *bb *ab *bb *bb *bb*bb*bb*bb *bb *bb *bb *bb *bb *bb

**Fig 1 (c)**




*Gpi-1*:* *aa *aa *aa *aa *aa *aa *aa *aa *aa *aa *aa *aa *aa *aa *aa *aa *aa *aa *aa *aa *aa *aa *aa *aa *aa *aa *aa *aa *aa *aa *aa

*Gpi-2*:* *ab *ab *ab *aa *aa *aa *aa *aa *aa *aa *aa *aa *aa *aa *aa *aa *aa *aa *aa *aa *aa *aa *aa *aa *aa *aa *aa *aa *aa *aa

**Fig 1 (d)**

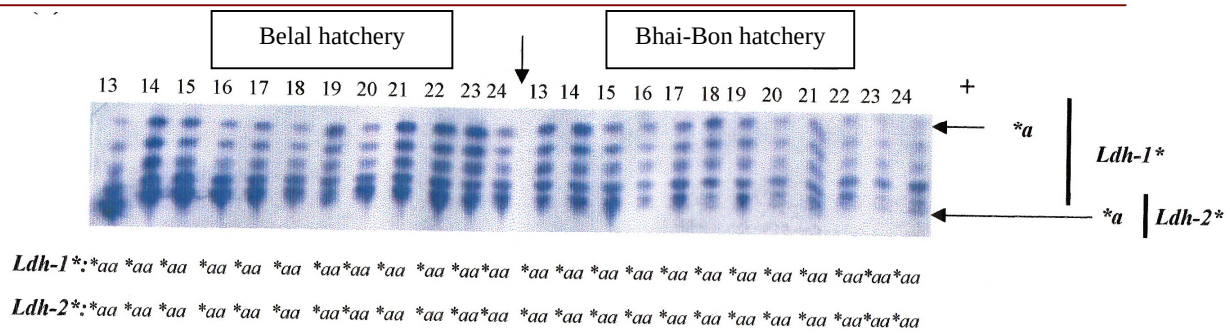



**Fig 1 (e)**

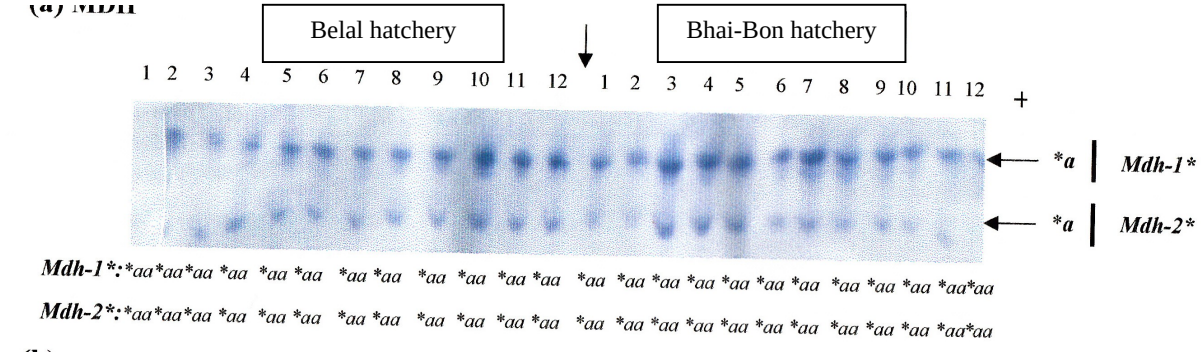



**Fig 1 (f)**

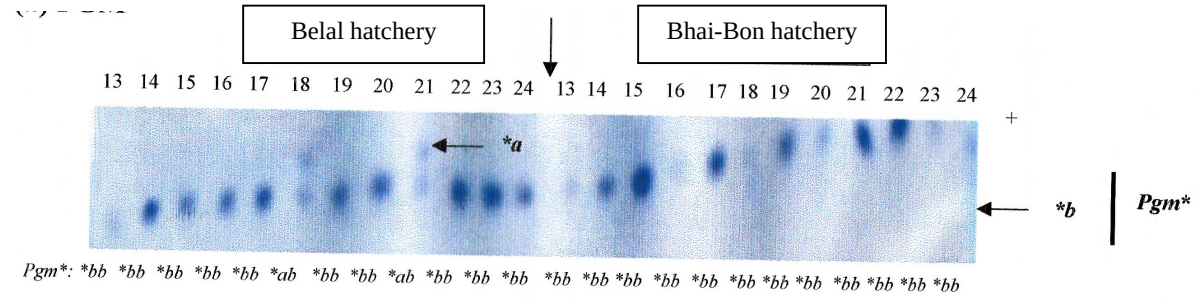



**Fig 1 (g)**

**Table.2** Enzymes examined and tissue used for allozyme electrophoresis

| Types of enzymes | Enzyme patterns | E.C. Number | Tissue |
|---|---|---|---|
| Alcohol dehydrogenase (ADH) | Dimer | 1.1.1.1 | Muscle |
| Aspertate aminotransferase (AAT) | Dimer | 2.6.6.1 | Muscle |
| Esterase (EST) | Monomer | 3.1.1.1 | Muscle |
| Glycerol-3-phosphate dehydrogenase (G3PDH) | Dimer | 1.1.1.8 | Muscle |
| Glucose-6-phosphate dehydrogenase (G6 PDH) | Dimer | 1.1.1.49 | Muscle |
| Glucose-6-phosphate isomerase (GPI) | Dimer | 5.3.1.9 | Muscle |
| Isocitrate dehydrogenase IDH(IDHP) | Dimer | 1.1.1.42 | Muscle |
| Lactate dehydrogenase (LDH) | Tetramer | 1.1.1.27 | Muscle |
| Malate dehydrogenase MDH | Dimer | 1.1.1.37 | Muscle |
| Phosphoglucomutase PGM | Monomer | 5.4.2.2 | Muscle |

**Table.3** Allele frequency at 11 presumptive loci of *Thai pangas*

| Locus | Allele | Allele frequency | | | |
|---|---|---|---|---|---|
| | | ***Belal*** | ***Bhai-Bhai*** | ***Bhai-Bon*** | ***Jahangir*** |
| Adh-1* | *a | 1.00 | 1.00 | 1.00 | 1.00 |
| Est-1* | *a | 0.38 | 0.87 | 0.43 | 0.78 |
| | *b | 0.62 | 0.13 | 0.53 | 0.22 |
| | *c | - | - | 0.03 | - |
| G3pdh-1* | *a | 0.20 | 0.08 | 0.07 | 0.07 |
| | *b | 0.80 | 0.92 | 0.93 | 0.93 |
| Gpi-1* | *a | 1.00 | 1.00 | 1.00 | 1.00 |
| Gpi-2* | *a | 0.58 | 0.60 | 0.72 | 0.87 |
| | *b | 0.42 | 0.40 | 0.28 | 0.13 |
| Idhp-1* | *a | 1.00 | 1.00 | 1.00 | 1.00 |
| Ldh – 1* | *a | 1.00 | 1.00 | 1.00 | 1.00 |
| Ldh – 2* | *a | 1.00 | 1.00 | 1.00 | 1.00 |
| Mdh -1* | *a | 1.00 | 1.00 | 1.00 | 1.00 |
| Mdh -2* | *a | 1.00 | 1.00 | 1.00 | 1.00 |
| Pgm* | *a | 0.63 | 0.37 | 0.58 | 0.52 |
| | *b | 0.37 | 0.48 | 0.38 | 0.37 |
| | *c | - | 0.15 | 0.03 | 0.12 |

**Table.4** Analyzed sample size and chi-square ($\chi^2$) test of fit to Hardy-Weinberg expectation

| Locus | | Population | | | |
|---|---|---|---|---|---|
| | | ***Belal*** | ***Bhai-Bhai*** | ***Bhai-Bon*** | ***Jahangir*** |
| Est-1* | $\chi^2$ | 6.478*[a] | 6.327*[a] | 2.430 | 3.371 |
| | df | 1 | 1 | 3 | 1 |
| G3pdh-1* | $\chi^2$ | 4.80*[a] | 0.195 | 0.113 | 8.816*[a] |
| | df | 1 | 1 | 1 | 1 |
| Gpi-2* | $\chi^2$ | 13.663*[a] | 10.845*[a] | 5.970*[a] | 37.793*[a] |
| | df | 1 | 1 | 1 | 1 |
| Pgm* | $\chi^2$ | 23.015*[a] | 39.542*[a] | 27.181*[a] | 42.005*[a] |
| | df | 1 | 3 | 3 | 3 |

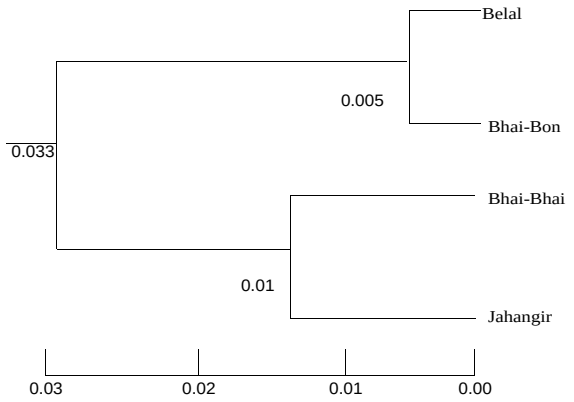
**Table.5** Genetic variabilities at 11 loci of *Thai pangas*

| Population | Mean proportion of polymorphic loci* per population | Mean proportion of heterozygous loci per individual (%) | Mean no. of alleles | Heterozygosity | | | |
|---|---|---|---|---|---|---|---|
| | | | | ***Ho*** | ***He*** | ***Ho/ He*** | **1- *Ho/ He*** |
| *Belal* | 36.36 | 13.33 | 1.364 | 0.103 | 0.162 | 0.636 | 0.364 |
| *Bhai-Bhai* | 36.36 | 13.33 | 1.455 | 0.061 | 0.137 | 0.445 | 0.555 |
| *Bhai- Bon* | 36.36 | 13.33 | 1.545 | 0.085 | 0.146 | 0.582 | 0.418 |
| *Jahangir* | 36.36 | 13.33 | 1.455 | 0.039 | 0.120 | 0.325 | 0.675 |

**Table.6** Genetic identities (above diagonal) and distances (below diagonal) among four populations of *Thai pangas* samples based on 11 loci

| Population | *Belal* | *Bhai-Bhai* | *Bhai- Bon* | *Jahangir* |
|---|---|---|---|---|
| *Belal* | - | 0.968 | 0.996 | 0.972 |
| *Bhai-Bhai* | 0.033 | - | 0.976 | 0. 990 |
| *Bhai- Bon* | 0.005 | 0.024 | - | 0.986 |
| *Jahangir* | 0.029 | 0.010 | 0.015 | - |

**Fig.2** UPGMA dendogram derived from Nei's genetic distance among four hatchery populations of *Thai pangas*




The average expected heterozygosity ($H_e$) for the populations was 0.141±0.018. The average $H_o$/ $H_e$ was found to be 0.497±0.14 for the populations and ranged from 0.325 (Jahangir population) to 0.636 (Belal population).

## Genetic differentiation

The coefficient of gene differentiation ($G_{ST}$) was estimated to be 0.614. The genetic distance (D) among four populations was found to range from 0.005 to 0.033. The minimum genetic distance was observed between Belal and Bhai-Bon populations while the maximum value (0.033) was found between the Belal and Bhai-Bhai populations.

In the present study, 10 enzymes were used in CA6.1 and TC-1 buffer systems for muscle tissue of Thai pangas where eight enzymes showed clear resolution. The unclear resolution of the other enzymes could account for the buffer system, tissue or species specificity.

Electrophoretic data have extensively been used for population genetics. The proportion of polymorphic loci is a commonly used measure of electrophoretically detectable variation in a population. The observed average proportion of polymorphic loci per population in this study was 36.36% which is higher than that of the reported average 22% in freshwater species like dace (Hanzawa et al., 1988) and 18% of 20 species of pangasiid catfish (Pouyaud et al., 1998) but much lower (65.22%) than that for Yellow Catfish *Mystus nemurus* (Leesa-Nga-SN et al. 2000). Na-Nakorn et al. (1998) found that the percentage of polymorphic loci (*P*) was between 10.5 and 26.3 in *Clarias macrocephalus.* The value of *P* in this study (36.36%) is higher than that

obtained by Barua et al. (2004) (19.4%). Nevo et al. (1984) estimated polymorphic loci as 15.2% ($p \leq 0.95$) for polymorphism in fish in general. Therefore the studied *Pangasius* population showed a good level of polymorphism in comparison to the above mentioned catfishes. The average heterozygous loci per individual obtained in the present study (13.3%) is close to the findings for the six populations (8.7-17.4%) of Indonesian common carp studied by Sumantadinata and Taniguchi (1990), higher than that obtained by Akanda (2001) for both hatchery and natural populations of *Catla catla* and lower than that (15%) reported by Alam (2002) for both hatchery and natural populations of *Labeo rohita*. The mean number of alleles per locus (1.455) as obtained in the present study was higher than that obtained by Na-Nakorn et al. (1998) (1.275) for *Clarias macrocephalus* and that obtained by Barua et el. (2004) for *P. hypophthalmus*. The average observed heterozygosity ($H_o$) estimated in the present study (0.072) was lower than that (0.091) reported by Pouyaud et al. (1998) but similar to that obtained by Na-Nakorn et al. (1998). The higher observed and expected heterozygosity ($H_o$=0.103 and $H_e$=0.162) exhibited by Belal population indicated that the gene pool of that hatchery was maintained effectively. The $H_o$ values indicated that those corresponding to the hatchery population of Thai pangas were closer to the average values obtained for teleosts ($H_o$=0.055, Kirpichnikov, 1992). Nevo (1978) reported an average observed heterozygosity value for bony fish 0.051. The level of heterozygosity is often related with the size of populations within a species. The species with small populations might lost variation due to genetic drift (Reina et al., 1994). The practical interest of higher heterozygosity value (Ho) of a population can be aimed at genetic breeding programmes. The average heterozygosity is considered as a good indicator of the genetic variability throughout the genome of the population (Leary and Booke, 1990; Allendorf and Ryman, 1986).

The Gst value (0.614) of the total population as obtained in the present study is lower than that obtained by Barua at el. (2004) (Gst = 0.792) for *P. hypophthalmus*. However, this Gst value suggests a larger proportion of genetic variation in the Thai pangas, possibly due to differences among populations from different hatcheries. The Gst value (0.614) of Thai pangas populations as obtained in the present study is lower than that obtained for other freshwater fishes such as loach (0.774) ( Khan and Arai, 2000) and freshwater Gobi (0.698) (Shimizu at el. 1993). However, the present study indicates that higher genetic differentiation among populations is maintained. Based on genetic distance (D-value), the four populations can be grouped into two as shown in the dendogram (Fig 2). First group comprised of Belal and Bhai-Bon population whereas second group comprised of Bhai-Bhai and Jahangir populations; the later was separated from the former by a genetic distance of D = 0.033. Furthermore, in first group, the Belal population was separated from bhai-Bon population at D = 0.005. In second group, the Bhai-Bhai population was differentiated from Jahangir population at D = 0.01. The observed genetic distances among the four populations of Thai pangas in the present study are much more lower than the findings of Pouyaud et al. (1998) who found the average distances within the species *P. polyuranodon* (D = 0.106) between population of Kalimantan and the population of Chao Phraya or *P. micronema* (D = 0.145) between population of Teluk Kuantan in Sumatra and population of sole in Japan. Leesa-Nga-SN et al. (2000) mentioned that the D-values of yellow catfish *Mystus nemurus* ranged from 0.005 to 0.164 and

suggested that the highest genetic distance among them was the subspecies level. Similar results were observed by Shimizu et al. (1993) and also suggested that the highest genetic differentiation among the five groups of *Rhinogobius* was the species or subspecies level. Nei (1972) found that in a variety of animals, D is approximately 1.0 for inter species comparisons, around 0.1 for subspecies, and 0.01 for local aces. Ayala (1975) reported that the D-value between subspecies is approximately 0.20. Considering from the above mentioned criteria, the studied *P. hypophthalmus* may be fallen into the local race or population. The studied hatchery populations were very closely located, but genetically Belal population showed greater variation. This may be due to either different sources of brood or using of the brood of various generations. Furthermore according to the result, the present genetic conditions of Bhai-Bhai and Jahangir population need to be improved. Genetic improvement of such hatchery populations can best be accomplished through the use of new brood from large broodstock and/or crossing with large gene pool. It is also emphasized that selective breeding programme should be rigorously followed for sustaining the original quality of such introduced fish species for long term conservation and management practices.

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