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Genetic variation in Hilsa shad *(Tenualosa ilisha)* population in River Ganges

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ABSTRACT

Genetic structure of the anadromous clupeid hilsa shad (*Tenualosa ilisha*) in Ganges was investigated using allozyme markers. A total of 26 loci are genotyped for samples collected during spawning period from five riverine locations *viz.* Brahmaputra, Padma, Ganga, Hoogly and Feeder canal. Despite the high level of polymorphism (31-50%, most common allele < 0.99), the samples from different locations do not exhibit significant genetic heterogeneity. The present results do not support earlier reports of the possibility of different races of hilsa in Ganges, but provide evidence for the existence of a single panmictic population. The observed homogeneity of allele frequencies is most likely to be explained by gene flow between the hilsa populations ascending different channels of Ganges.

Introduction

Tenualosa ilisha inhabits coastal shelf, estuaries and fresh water rivers in Indonesia, Sumatra, Myanmar, Bangladesh, India, Pakistan, Kuwait, Iraq and Iran. Hilsa is anadromous, reported to ascend rivers as far as 1200 km inland for breeding (Pillay & Rosa, 1963) and after spawning in freshwater returns to marine habitats.

Ganga river system joined with Brahmaputra river is one of the largest drainage used by hilsa. Migration of hilsa in the Ganges occurs during breeding season. Hilsa sustained a lucrative fishery in the middle strecth of Ganga which declined after the commissioning of Farrakka barrage in 1972 (Sinha, 2000).

Presence of more than one race of hilsa has always evoked great interest among researchers (Chonder, 1999). Majumdar (1939) recognised three ecotypes of hilsa, from saline water of the sea, muddy fresh water and from clear fresh water. Using biometrical comparisons, Pillay *et al.* (1963) delineated three stocks of hilsa with different distribution. Based on morphological differences, slender and broad morphotypes of hilsa have been identified in the Ganges (Ghosh *et al.*, 1968, Quddus *et al.*, 1984). Malvin (1984)

opined that hilsa in Bay of Bengal may represent assemblage of heterogenous groups from different rivers in India, Bangladesh and Myanmar which return to their natal rivers for spawning. Existence of permanent riverine populations of hilsa in Ganga has also been suggested (Chonder, 1999, Fish Base, 2000). Recent tagging experiments proved that anadromous population of hilsa in the River Padma migrates across Farraka barrage and is responsible for recruitment to population and limited fishery in River Ganga above barricade (Anon., 1996). The studies also suggested inability of the fish to ascend from Hoogly - Bhagirathi to Ganga due to obstruction but downward movement of brooders and juveniles exists. The tagging experiments do not support earlier reports of Pillay *et al.* (1963) that there is little or no movement of hilsa between Hoogly, Padma and Ganga Rivers and little intermingling of the populations.

At present, understanding of genetic variation and divergence in hilsa population across its range of distribution is limited. Dahle *et al.* (1997) and Rahman and Naevdal (2000) found that hilsa in marine water is genetically different from that in freshwater and brackish waters in Bangladesh using RAPD and allozyme markers, respectively. Significant differences were reported in RAPD profiles between fresh and brackish water collections, though inference from allozyme variations only partly supported. Another study using allozyme markers suggests that hilsa in Bangladesh belong to only one population including collections from riverine locations and Bay of Bengal, while significant deviations are observed in collections from Kuwait and Indonesia (Hussain *et al.,* 1998, BFRI, 2001). Otolith microchemistry also support results from these genetic studies and suggest that hilsa in Bay of Bengal, need to be managed as a single stock (Milton and Chenery, 2001).

The present investigation analyses allozyme variability to explore delineation of genetic divergence in hilsa population along the River Ganges and associated rivers. In addition genetic variation due to anadromous stocks of hilsa ascending through western end of Gangetic delta i.e., the Hoogly-Bhagirathi river system, is compared with samples from middle stretch of Ganga where fishery is known to have declined. The study is aimed at providing data for application in stock based fisheries management and restoration in planning besides obtaining new data to consider its endangered status.

Materials and methods

The main channel of the River Ganga after Farrakka barrage flows into Bangladesh as river Padma and meets the River Brahmaputra (Jamuna), thereafter the River Meghna joins before draining into Bay of Bengal through Meghna estuary. Upstream to Farrakka barrage, the Ganga is linked to the river Bhagirathi through 41 Kms long manmade feeder canal that in turn joins the Hooghly to drain into the Bay of Bengal. Gangetic delta is bound by estuaries, Meghna (eastern end) and Hoogly (western end) and is characterized by several intermittent channels like Jalangi, Churni (ECAFE, 1966). Samples of hilsa were collected during the spawning period through commercial boat seine operations from the Ganges at five riverine (Table 1) locations *viz.*, Brahmaputra (Guwahati), Padma (Baniagram), Ganga (Allahabad, Varanasi), Hooghly (Kolkatta) and Feeder canal (Farrakka). The fish were dissected at site and liver samples

TABLE 1. *Sample size of* Tenualosa ilisha *and sampling period at five riverine locations.*

River system (Location)	Total sample (N)	Sampling date	Sample size (n)
Hoogly (Calcutta)	110	Sep. 1999	35
		Oct. 1999	50
		Nov. 1999	25
Padma			
Down Farraka	90	Sep. 1999	50
barrage(Beniagram)		Nov. 1999	40
Ganga			
above Farraka	06	Oct. 1997	50
barrage			
(Allahabad)	05	Nov. 1999	40
(Varanasi)			
Feeder	50	Oct. 1999	40
canal/Bhagirathi		Nov. 1999	10
(Farrakka)			
Brahmaputra (Guwahati)	29	Feb. 2000	29

excised and immediately immersed in liquid nitrogen (-196°C). The samples in frozen state were transported to laboratory and held at -80^0 C until use. The hilsa were in running ripe or advanced stages of gonadal maturation, including samples collected from Brahmaputra. Collection during breeding is important for getting true picture of genetic differentiation in a species migrating for spawning.

The liver pieces were crushed mildly in cooled microcentrifuge tubes with four volumes of extraction buffer (0.17 M Sucrose, 0.2 M EDTA, 0.2 M Tris-HCL, pH 7.0). Suspensions were centrifuged for 1 hour at 10, 000 rpm at 4^{0}C and 200 ml of supernatant was again centrifuged for 20 min. Allozyme variations were investigated using vertical electrophoresis units (gel size 8 x 7 cm, Hoeffer Scientific mighty small SE 250) through 7% polyacrylamide gels (Gopalakrishnan *et al.*, 1997). Electrophoresis runs were conducted at constant voltage of 150 V in Tris-Borate Buffer (500 mM Tris, 650 mM Boric Acid, 16mM EDTA, pH 8.0). The choice of enzyme systems, tissue, buffer and running conditions were based on our previous work on hilsa shad (NBFGR, 1998). The allozyme profiles were visualized using histo-chemical staining methods adopted from Whitmore (1990). The nomenclature for loci and alleles were done as recommended by Shaklee *et al.* (1990). At all loci, the most common allele (found in Padma samples) was assigned as 100. Alternate alleles were designated as per their mobility relative to the most common allele. Temporal stability of allele frequencies in hilsa collections from River Padma for two years has also been observed in our earlier study (NBFGR, 1998).

The data were analysed through the software programmed Genetix 4.0 (Belkhir *et.al.,* 1998) for estimates of classical variables of polymorphism and F_{st} with statistical significance. Options in Genepop 3.1d (Raymond and Rousset, 1995) were used for performing

probability test for Hardy-Weinberg equilibrium and finding population differentiation (genotypic and allelic). The individual genotype data were analysed for genotypic differentiation between samples collected during different periods within each location. The data sets were combined within a site if no significant differences were observed. Conformity to Hardy-Weinberg expectations were tested for each collection set as well as combined data set. Differentiation among different locations was assessed with the combined data set. The classical variables like heterozygosities, mean number of alleles per locus and proportion of polymorphic loci (most common allele < 0.95 & 0.99) were calculated for all the locations. Tests for allelic and genotypic differentiation were performed for each locus for all pairs of locations. Wright's F_{st} (Wright, 1978) was used to characterize genetic sub-division based on proportion of total genetic variation due to differences between populations. Multilocus estimate of F_{st} between all pairs of locations was calculated as estimator q (Weir and Cockerham, 1984) and statistical significance was derived using a permutation test (No. of permutation = 1000).Significance levels were adjusted to account for multiple tests through sequential bonferroni method (Lessios, 1992). The probabilities were arranged in descending order and adjusted significant level for individual test probability is 0.05/ n+1. Here, 'n' is the no. of tests preceding the test probability. All the tests falling after the probability level that is found significant are considered significant.

Results

Fifteen enzyme systems were examined which produced twentysix consistently scorable loci (Table 2). Thirteen loci were polymorphic at 99 percent criteria (most common allele < 0.99, Table 3). The alternate allele 123 (in Padma samples) found at locus MDH-2 * has very low frequency (< 0.01), hence locus MDH-2 * was not included in the

TABLE 2. *Enzymes, loci scored to examine genetic structure in* Tenualosa ilisha

Enzyme	Enzyme Commission Number	Locus
Acid phosphatase	3.1.3.2	*ACP**
Aspartate amino transferase	2.6.1.1	*AAT-2*, AAT-3**
Esterase	3.1.1. -	*EST-I*, EST-2*, EST-3* EST-4*, EST-5**
Glucose-6-phosphate dehydrogenase	1.1.1.49	*G6P D-I **
Glucose phosphate isomerase	5.3.1.9	*GPI**
Glutamate dehydrogenase	1.4.1.3	*GLUD**
a-glycerophosphate dehydrogenase	*1.1.1.8*	*GPD-3**
Isocitrate dehydrogenase	1.1.1.42	*IDH**
Lactate dehydrogenase	1.1.1.27	*LDH-I*, LDH-2*, LDH-4**
Malate dehydrogenase	1.1.1.37	*MDH-2**
Malic enzyme	1.1.1.40	*ME-I*, ME-2*, ME-3**
Phosphogluconate dehydrogenase	1.1.1.44	*PGDH**
Phosphogluco mutase	5.4.2.2	*PGM-2*, PGM-3**
Superoxide dismutase	*1.15.1.1*	*SOD-I*, SOD-2**
Xanthine dehydrogenase	*1.1.1.204*	*XDH**

analysis. The genotype distribution at the polymorphic loci did not exhibit significant departures (Benferroni correction applied, P > 0.05) from those expected under Hardy-Weinberg equilibrium, indicating that basic assumption of random mating is not violated. The genotype distribution of samples collected at different months within the same locations did not exhibit heterogeneity (P > 0.05). This allows combining of data to produce five sets, each for one riverine locations *viz.*Hoogly, Bhagirathi, Padma, Ganga and Brahmaputra for subsequent analysis.

The parameters of genetic variation are given in Table 4. The mean heterozygosity per locus ranged between 0.134 (Brahmaputra) and 0.154 (Ganga). Mean heterozygosity over all the populations is 0.143. Mean number of alleles per locus ranged from 1.346 (Ganga) to 1.615 (Padma). Pairwise comparisons between different riverine locations for allelic homogeneity did not yield significant deviation at any locus in their allele frequencies after significance level are adjusted for Bonferroni corrections. The estimate of F_{st} did not differ significantly (P > 0.05) from zero for any pair of locations (Table 5).

Discussion

Allozyme markers provide evidence of high level of genetic variation (31- 50% loci polymorphic, most common allele < 0.99) in hilsa, but do not exhibit genetic divergence among samples. This can be attributed to wide distribution of hilsa along with high dispersal capability due to anadromous life strategy. Anadromous populations tend to have more genetic variation but less genetic differentiation than landlocked or fresh water resident populations (Ryman, 1997). This results in an increase in the probability of an individual migrating to other sub-populations and exchanging genes, thus retarding divergence. Negative correlation between dispersal capability and G_{st} (Coefficient of genetic differentiation) has been demonstrated by Waples (1987). Schribner *et al.* (1998) reported low population differentiation (q = 0.01) over eighteen polymorphic loci (Ho = 0.250) in chum salmon *(Oncorynchus keeta)* populations of Yukon River. Similarly genetic differentiation was not observed in various habitats in Hudson River for American shad (Waldman *et al.,* 1996) and gizzard shad in Mississippi River (Hartfield *et al.*, 1982). Different morphotypes of hilsa classified by earlier workers (Pillay *et. al.,* 1963; Ghosh *et.al.*, 1968; Quddus *et al.*, 1984) could have been due to phenotypic plasticity or environmental influence (Swain and Foote, 1999). Morphological variations have been reported in otherwise genetically homogenous populations of clupeid species of Spanish sardine (Kinsey *et al.,* 1994) and European anchovy (Tudela, 1999).

The present study do not provide evidence of genetic divergence in hilsa populations from the stretch of Ganges under study. Allele frequencies do not deviate at any locus between the samples from Brahmaputra and Padma and belong to same genetic stock. It is likely that hilsa from same genepool ascends from Bay of Bengal through Meghna estuary to river Padma and portion of it progresses towards Brahamaputra (called as Jamuna in Bangladesh). Further, the fish ascending to river Padma must have the opportunity of mixing and spawning with the hilsa in - Bhagirathi system so as to explain the observed insignificant genetic differentiation between samples from

TABLE 3. *Allele frequencies for thirteen polymorphic loci in* Tenualosa ilisha *from five riverine locations.*

Locus	Riverine locations				
	Hoogly	Padma	Feeder canal/ Bhagirathi	Ganga	Brahmaputra
(N)	108	89	48	11	29
AAT-3*					
100	0.9210	0.9045	0.9375	1.0000	0.9655
122	0.0880	0.0955	0.0625	0.0000	0.0345
*EST-2**					
94	0.0694	0.0506	0.0521	0.0000	0.1724
98	0.2593	0.2584	0.2083	0.3278	0.1552
100	0.4398	0.4944	0.5104	0.4766	0.6034
106	0.2315	0.1966	0.2292	0.1956	0.0690
*EST-3**					
100	0.6667	0.6461	0.6458	0.6821	0.6034
102	0.3333	0.3539	0.3542	0.3179	0.3966
EST-4 *					
93	0.3889	0.3258	0.3958	0.3358	0.2586
100	0.6111	0.6742	0.6042	0.6642	0.7414
*GPD-3**					
87	0.0046	0.0000	0.0000	0.0000	0.0000
100	0.9815	0.9663	0.9479	1.0000	0.9828
109	0.0139	0.0337	0.0521	0.0000	0.0172
*GP-I**					
81	1.0000	0.9888	1.0000	1.0000	1.0000
100	0.0000	0.0112	0.0000	0.0000	0.0000
*LDH-4**					
100	1.0000	0.9888	0.9896	1.0000	1.0000
125	0.0000	0.0112	0.0104	0.0000	0.0000
*MDH-2**					
100	1.0000	0.9944	1.0000	1.0000	1.0000
123	0.0000	0.0056	0.0000	0.0000	0.0000
*ME-2**					
100	0.4861	0.5281	0.4375	0.4461	0.4828
118	0.5139	0.4719	0.5625	0.5539	0.5172
*PGDH-1**					
100	0.6250	0.6011	0.6458	0.6238	0.6724
103	0.3750	0.3989	0.3542	0.3762	0.3276
*PGM-2**					
66	0.4491	0.3427	0.4167	0.4864	0.3621
100	0.5509	0.6573	0.5833	0.5136	0.6379
*PGM-3**					
100	0.9074	0.8933	0.9167	0.8921	0.9483
141	0.0926	0.1067	0.0833	0.1079	0.0517
*SOD-2**					
100	0.9769	0.9719	1.0000	1.0000	1.0000
137	0.0231	0.0281	0.0000	0.0000	0.0000
*XDH**					
100	0.5741	0.5449	0.6146	0.5972	0.5862
108	0.4259	0.4551	0.3854	0.4028	0.4138

TABLE 4. *Genetic variation in* Tenualosa ilisha *at twenty six loci in samples from five riverine locations.*

River	Mean heterozygosity		Polymorphic loci (%)		Mean no. of alleles / locus
	H (HW exp.)	H (obs.)	P < 0.95	P < 0.99	
Hoogly	0.152	0.145	34.62	42.31	1.539
Padma	0.154	0.145	34.62	50.00	1.615
Feeder canal / Bhagirathi	0.149	0.135	38.46	42.31	1.500
Ganga	0.140	0.154	30.77	30.77	1.346
Brahmaputra	0.136	0.134	30.77	38.46	1.462

TABLE 5. F_{st} (above diagonal) and probability values (below diagonal) of multi locus genotype data for pairs of riverine locations.

River	Hoogly	Padma	Feeder can / Bhagirathi	Ganga	Brahmaputra
Hoogly	0	0.0009	-0.0049	-0.0270	0.0073
Padma	0.430	0	-0.0011	-0.0185	0.0008
Feeder canal / Bhagirathi	0.956	0.540	0	-0.0205	-0.0011
Ganga	0.907	0.769	0.749	0	0.0116
Brahmaputra	0.089	0.391	0.428	0.287	0

distant locations like Brahmaputra and Hoogly. It is emphasized that the observed homogeneity of allele frequencies between hilsa populations in Ganges may not be possible without the geneflow between the fish ascending from two extremes of delta *viz.* Meghna estuary and Hoogly estuary.

The mixing of migratory stocks is likely to occur; i.) analysing retrospectively, when Farrakka barrage was not an obstruction, hilsa originating from Hoogly and Meghna would have been migrating above Farrakka into single channel i.e. Ganga. Geneflow of much larger magnitude than today, between the putative sub-populations could have been possible; ii.) through channels (e.g. Jalangi and Churni) hence providing continuous link between Padma and Hoogly -Bhagirathi system during rains; iii.) downstream movement of juveniles as well as brood fish from Ganga to Bhagirathi - Hoogly through man-made feeder canal (Anon, 1996) and iv) tagging experiments confirmed the movement of brooders across Farrakka barrage from River Padma to Ganga and recruitment above Farrakka from migratory stock (Anon, 1996).

It can be inferred that consistent geneflow does not allow genetic drift to cause genetic differentiation in hilsa population and does not support the possibility of hilsa returning from Bay of Bengal to natal rivers for spawning, as opined by Malvin (1984).

These conclusions are consistent with the results from allozyme studies carried on hilsa from Ganges in Bangladesh (Rivers Padma and Meghna). No evidence of genetic heterogeneity has been reported in Bangladesh samples and the genepool is

shared with that in Bay of Bengal (Hussain *et al.,* 1998, BFRI, 2001). Milton and Chenery (2001) found this genetic evidence in conformity with the results from otolith microchemistry and concluded that Bay of Bengal from south East India to Myanmar through Bangladesh harbours the same genepool of hilsa. However, this argues against the likelihood of more than one genetic stock of hilsa in Ganges and Bay of Bengal as reported by Rahman and Naevdal (2000).

Given the present findings from available genetic studies on hilsa and other anadromous fish species without homing fidelity, it is unlikely that significant genetic differences will be detected in hilsa population within Ganges and associated rivers. In the absence of stock differences in hilsa population in Ganga River system especially between above (Ganga) and below Farrakka barrage (Padma and Bhagirathi- Hoogly), inclusion of this species among threatened fishes is not justified. Hilsa catches in Hoogly River was also reported to have increased consistently up to more than three folds in post Farrakka barrage period (Sinha *et al.,* 1998). However, the real concern is to evolve strategy for successful revival of fishery through artificial propagation and restoration in the ranges where abundance is declined like upstream of Farrakka barrage and other places. In the light of present findings, it will be possible to use the progeny produced from brood fish belonging to Rivers Padma and Hoogly for ranching without dangers associated with mixing of stocks.

For effective rehabilitation process, sound technology for breeding, rearing as well as ranching is prerequisite. Artificial propagation of hilsa has been partially successful in India (De and Sinha, 1987; Sen *et al.,1990;* Talwar and Jhingran, 1992; Chonder, 1999). Spermatozoa cryopreservation technique (Lal *et al.,* 1999) can be a useful tool in breeding procedures. Genetic diversity revealed through polymorphic microsatellite markers has proved effective in brood stock analysis and parentage determination of hatchery fry for propagation assisted restoration of nearly extinct American shad in James River (Brown *et. al.,* 2000). The polymorphic markers and data generated in this study can be useful as baseline for monitoring the impact of ranching on genetic variability in populations.

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