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RAPD ANALYSIS OF HILSA, *TENUALOSA ILISHA* IN THE BRAHMAPUTRA RIVER SYSTEM DURING MIGRATION

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ABSTRACT

The andromous migratory shad Hilsa,*Tenualosailisha* is a migratory fish migrates from Bay of Bengal to firewater Brahmaputra. RAPD analysis was done for the differentiating the genetic strain of different hilsa populations. RAPD is a type of PCR reaction, but the segments of DNA that are amplified are random. For performing RAPD analysis first creates several arbitrary, short primers, and then proceeds with the PCR using a large template of genomic DNA, that fragments will amplify. Eye lens of four Hilsa samples from different areas following protocol as mentioned in "Spin Mammalian Genomic DNA Prep" kit (Genei Catalogue # KT 82). Performed RAPD reactions using RAPD primers (RHU-1 & RHU-2 used separately) under PCR conditions. All samples from different localities show polymorphism with Primer RHu1 and RHu2. The RAPD fingerprinting was scored for the presence and abundance of fragments on the gel photographs and the RAPD fragments were compared among the four Hilsa populations *i.e.* Dhubri, Goalpara, Guwahati and Tezpur. RAPD banding patterns in Pixel intensity (Pi) were recorded using Gel Documentation Centre.

Key Words : Hilsa, *Tenualosailisha*, RAPD, DNA, Brahmaputra

INTRODUCTION :

Hilsa is an anadromous fish occurring in the Indo-west Pacific region from the Persian Gulf, along the coast of Pakistan, India, Bangladesh, and Burma to South Vietnam [1](Rahman and Naevdal, 1998; [2]AL-Hassan, 1982). Hilsa is an important tropical fish belonging to the family Clupeidae and sustains a highly commercial fishery in the Brahmaputra river system in India. All Hilsa stocks appear to breed in the upper reaches where eggs, larvae and juveniles are found during the spawning seasons. During the breeding season they ascend the rivers and after spawning return to the original habitat where they remain until the next breeding season [3](Pillay and Rosa, 1963).

The upstream migration during the main breeding season appears to depend largely on the commencement of the southwest monsoon and consequent flooding of the rivers. The variations in the intensity of monsoon during the breeding season appear to cause considerable fluctuations

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in the abundance of the fish and catches in different places. In India, Hilsa distribution has been recorded from the Narmada and Tapti Rivers and from the Vembanad backwaters of western India. In the eastern region Hilsa is distributed in the Cauvery, Krishna, Godavari, Mahanadi, Hooghly, and Ganga Rivers [4](Chonder, 1999). In 1873, Day described two classes of Hilsa from the rivers, (a) one-year-old Hilsa appearing not to breed and (b) those breeding at the start and during the monsoon. [5]Jenkins (1938) raised the question whether two or more Hilsa races or varieties exist with different spawning grounds. [6]Mojumdar (1939) recognized three ecotypes of Hilsa from saline water of the sea, muddy freshwater and clear freshwater.

[7]Pillay *et al.*,(1963) differentiated three stocks of Hilsa using biometrical studies. Based on morphological characteristics, [8]Ghosh *et al.*,(1968) and [9]Quddus*et al.*,(1984) differentiated Hilsa into slender and broad morphotypes. [7]Pillay *et al.*,(1963) concluded that the Hilsa populations of the Hooghly, Padma and Ganga show little or no movement between the rivers, with little intermingling of populations. [10]Dahle*et al.,* (1998) used random amplified polymorphic DNA (RAPD) and discriminated between three different populations of Hilsa. Similarly, [11]Shifat*et al.*,(2003) used RAPD to differentiate the Padma and Meghna River Hilsa populations into two genetically different stocks or races. Thus, to gain insight into the structure of Hilsa populations, the RAPD technique was used to delineate populations from their spawning habitats in Indian rivers.

RAPD is a stripped-down version of PCR which uses a single sequence in the design of the primer (*i.e.* two primers are still needed for PCR: the same primer is used at either end). The primer may be designed specifically, but could be chosen randomly and is used to amplify a series of samples which will include both the material of interest as well as other control samples with which the experimental material needs to be compared. The choice of primer length will be critical to the determination of band complexity in the resulting amplification pattern. Eventually a particular probe will be found that is able to distinguish between the sample of interest (*eg.* a pit-bull terrier) and those that are different (*eg.* a Staffordshire bull terrier). While the RAPD method is empirical, its simplicity of use and the eventual identification of some stretch of DNA, albeit unknown, to facilitate discrimination, makes it a popular means of identifying breeds.

MATERIALS AND METHODS :

The muscle, liver and eye lens samples of Hilsa (N = 72) were collected from four different locations from the Brahmaputra River during March 2017 to April 2018. The tissue samples were preserved in 90% ethanol and genomic DNA was extracted following the [12]Sambrook and Russel (2001) protocol with some modifications. Random Amplification of Purified DNA (RAPD) analysis was followed by “Spin Mammalian Genomic DNA Prep” kit (Genei Catalogue # KT 82) using RAPD primers (RHU-1 & RHU-2) under PCR conditions.

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Protocol :

- Genomic DNA was isolated from eye lens of four Hilsa (*Tenualosailisha*) samples (*viz.*, A-Collected from Dhubri, B- collected from Goalpara, C-collected from Guwahati and D-collected from Tezpur) following protocol as mentioned in "Spin Mammalian Genomic DNA Prep" kit (Genei Catalogue # KT 82)
- Performed RAPD reactions using RAPD primers (RHU-1 & RHU-2 used separately) under PCR conditions as mentioned:

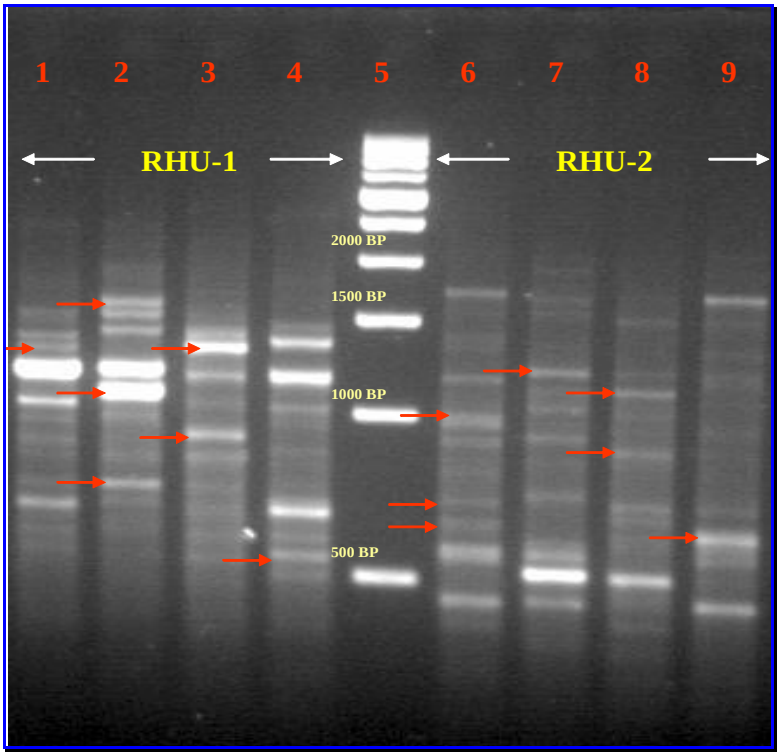
RESULTS AND DISCUSSIONS :

RAPD analysis was attempt in order to valididated the occurrence of 4 distinct population as has been observed during the length-weight study of the fish from different collecting stations.

Table-1. Cycle condition (For RAPD primer RHU-1 & RHU-2)

94°C	94°C	35°C	72°C	94°C	40°C	72°C	72°C
7 min	45 sec	60 sec	90 sec	45 sec	60 sec	60 sec	7 min
	8 cycles			35 cycles			

Fig-. 1. RAPD Profile of Hilsa samples Generated using Genei RAPD primer RHU-1& RHU-2 (1.5 agarose gel photo) :



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Primer sequences:

- ✓ RAPD Primer RHU-1: 5'- GAG GGC GAT C -3'
- ✓ RAPD Primer RHU-2: 5'- GAG GGC GAC T -3'
- ✓ 10 µl of PCR products were loaded on to 1.5 % agarose gel and resolved by electrophoresis. The gel was subsequently stained with ethidium bromide and viewed under UV-light. Photographs were documented subsequently (attached).
- ✓ Polymorphic DNA bands are marked by arrows (⟶)

Lane Description:

Lane 1: RAPD profile of sample A (Primer Used: RHU-1)
Lane 2: RAPD profile of sample B
Lane 3: RAPD profile of sample C
Lane 4: RAPD profile of sample D
Lane 5: **500 bp Ladder** (Genei™)
Lane 6: RAPD profile of sample A (Primer Used: RHU-2)
Lane 7: RAPD profile of sample B
Lane 8: RAPD profile of sample C
Lane 9: RAPD profile of sample D

Table- 2. Results for sample examined under primer RHu 1

Sample	No. Of bands scored	**No of unique bands**	**Approx band size (bp)**	**Primer**
Dhubri	8	1	1300	RHu 1
Goalpara	7	3	1600, 1100, 700	
Guwahati	7	2	1400, 900	
Tezpur	9	1	400	

Table- 3. Results for sample examined under primer RHu 2

Sample	No. Of bands scored	**No of unique bands**	**Approx band size (bp)**	**Primer**
Dhubri	8	3	1500, 600, 650	

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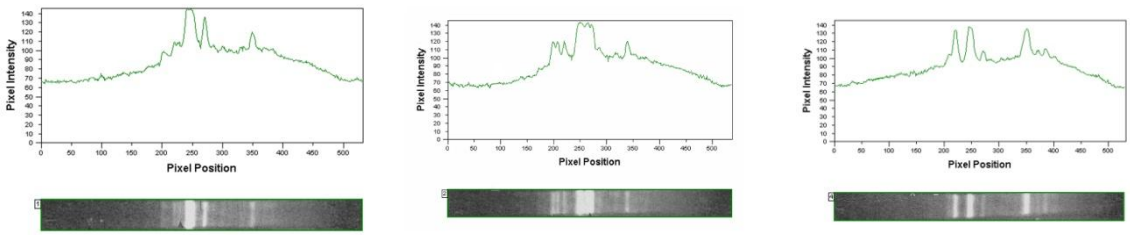
Goalpara	10	1	120	RHu 2
Guwahati	7	2	1050, 800	
Tezpur	7	1	570	

Pixel intensity :

Different pixel intensity of Hilsa sample are as follows :

Table 4. : Pixel intensity of the Hilsa.

Band Group	1	2	3	4	5	6	7	8	9	10
A	101	112	145	135	120					
B	119	120	121	143	144	140	119			
C	135	128	124	126	112					
D	134	138	110	135	109	111				
E	101	91	100	105	107	107	118	116		
F	90	89	104	95	98	100	101	120	144	115
G	85	88	103	104	106	105	130			
H	109	89	90	98	120	114				



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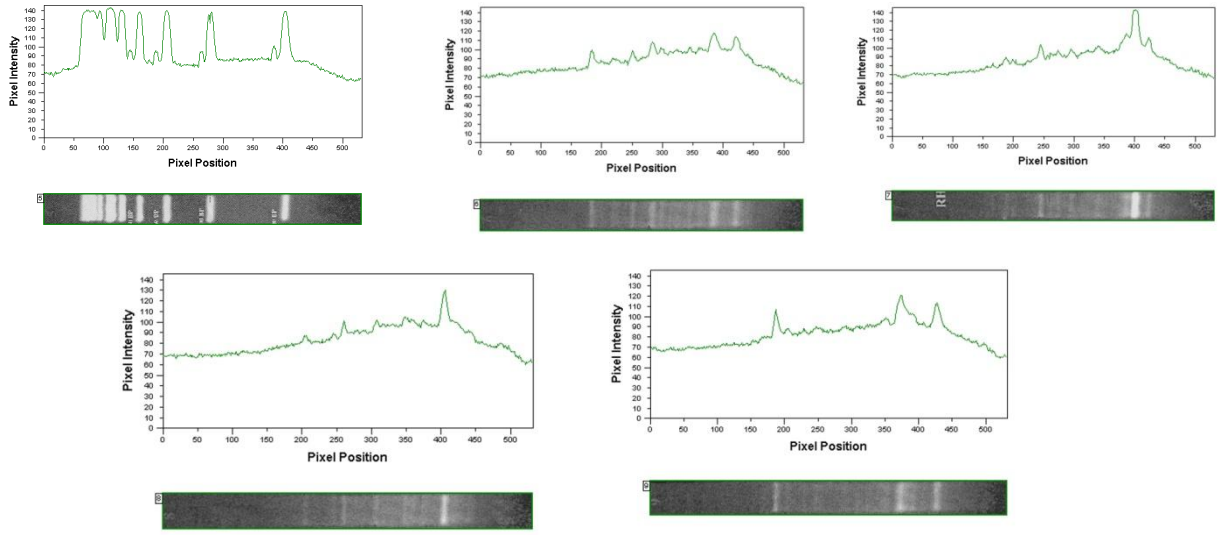

From Tables-1, 2 and 3. it is clear that one band is unique for Sample A, three bands are unique for Sample B, two bands for Sample C and one band for sample D with Primer RHu 1 (Fig-1).Three bands are unique for Sample A, one band is unique to Sample B, Two bands are unique to Sample C and one band is unique to Sample D with Primer RHu 2. All four samples show polymorphism with Primer RHu1 and RHu2.

Hilsa fishery is a commercially important fishery in Brahmaputra river system. The RAPD analysis has discriminated the four populations of Hilsa from the rivers draining into Dhubri in the eastern region of Brahmaputra. The RAPD fragments observed in the 72 individuals show polymorphism within and between the populations. The population specific bands could not be discerned from the fragment patterns generated. The cluster diagram has delineated the four different populations into two major clusters, which could be due to different spawning grounds of Hilsa. [10]Dahle*et al.*,(1998) used RAPD to differentiate three populations collected from the saline, brackish and freshwater regions of Bangladesh. The mean estimated similarity between Chandpur/Cox's bazar was 0.403 ± 0.070, Chandpur/Barguna was 0.419 ± 0.072 and Cox's bazar/Barguna was 0.409 ± 0.067. The average mean similarity between all these locations was 0.410 ± 0.069, which is similar to present findings. Similarly, [11]Shifat*et al.*,(2003) used RAPD to discriminate between the Hilsa populations from the Padma and Meghna Rivers. The Padma populations were genetically distant from the Meghna populations and within population variability in Padma and Meghna populations was also established. The dendrogram showed two major clusters for the Padma and Meghna Rivers, indicating the existence of two different spawning populations from the Padma and Meghna Rivers, respectively.

The present study shows the existence of genetic variation within and between the Hilsa populations of four inland rivers of India, indicating the presence of separate stocks or races of Hilsa that may be due to the river ecology, spawning grounds, nursery grounds of the juveniles, seasonal migration, and homing behavior of anadromous clupeids. The overall Fst found in Hilsa falls within the range reported for other migratory fish species (0.56 for Gulf and 0.44 for striped

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bass; [13]Bielawski*et al.*,1995). The extensive geographic distribution, long distance migrations, anadromous life histories, and strong homing instincts of anadromous fishes are major determinants in the population structuring of Hilsa. Once the population structure is known, scientific management for optimal harvest and conservation of the Hilsa fishery resource can be undertaken. In capture fishery, high exploitation combined with poor fishery management results in depletion of the fishery stocks. There are many examples [14](Begg and Waldmandel, 1999) viz. anchovy *Engraulisringens*[15](Hilborn and Walters, 1992), capelin *Mallotusvillosus*[16](Tjelmeland and Bogstad, 1993), Atlantic cod *Gadusmorhua* orange roughy*Haplostethusatlanticus*[17](Smith *et al.*,1991), and sardine *Sardinopssagax*[18](Shannon *et al..*, 1999). Such depletions can result in the loss of the total gene pool [19](Nelson and Soule, 1987; [16]Smith *et al..*, 1991). Disregard of stock structure in fisheries management can also lead to significant changes in the biological characteristics of a fish species [20](Altukhov, 1981; [21]Ricker, 1981).

The pixel intensity for each population also shows a polymorphic relationship among each other. The Dhubri fish shows 5 numbers of banding groups while in case of Goalpara it shows 7 nos. The Pi also varies different as at a group it varies as 101 and 119, at b-112 and 120, at c-145 and 121, at d-135 and 143, at e- 120 and 144 respectively.

The RAPD fingerprinting were scored for the presence and abundance of fragments on the gel photographs and the RAPD fragments were compared among the four hilsa populations *i.e*. Dhubri, Goalpara, Guwahati and Tezpur. RAPD banding patterns in Pixel intensity (Pi) were recorded using Gel Documentation Centre.

The Pi were observed (Table-4) that Hilsa of Dhubri can be categories into four groups 101, 112-120, 135 and 145 of RHU – 1 whereas in RHU – 2 it can be grouped into two groups (91, 100, 101, 105 and 107) and (118, 116). The Pi for Goalpara is categories into Pi 2 groups (119-112 and 140-144 of RHU-1 and RHU-2 into 4 groups: 89-98, 100-104, 115-120 and 144. The Pi for Guwahati in RHU-1 can be grouped into 112; 124-128 and 134 while RHU-2 can be categorized into 3 groups like 85-88; 103-106 and 130. The Pi for Tezpur is grouped for RHU-1 into 2 parts 109-111 and 134-138 while RHU-2 contains 89-98 and 109-120.

The two primers RHU-1 and RHU-2 were used to perform the amplification reactions. The primers generate a huge number of bands i.e. (9 bands). The similarity index (SI) values between populations were calculated as the average similarity randomly paired individual from populations RAPD profiles were calculated using the formula :

$SI = 2N_{AB}/ (N_A + N_B)$

Where, N_{AB} = The total number of RAPD bands shared by individuals A and B.

N_A and N_B = Number of bands scored for each individual respectively.

Primer RHU – 1 :

SI for Dhubri and Goalpara = 1.6
SI for Dhubri and Guwahati = 1.7
SI for Dhubri and Tezpur = 1.3
SI for Goalpara and Guwahati = 1.7

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SI for Goalpara and Tezpur = 1.6
SI for Guwahati and Tezpur = 1.4

Primer RHU – 2 :
SI for Dhubri and Goalpara = 1.9
SI for Dhubri and Guwahati = 1.9
SI for Dhubri and Tezpur = 1.7
SI for Goalpara and Guwahati = 2.0
SI for Goalpara and Tezpur = 2.1
SI for Guwahati and Tezpur = 1.9

The present RAPD study confirmed that there are more than one genetically isolated strain, which showed that there are 4 strains, while our earlier studies also it has been found that there are 4 strains sharing different length-weight relationship.

CONCLUSION :

RAPD technique was used to delineate Hilsapopulations from their spawning habitats in Indian rivers. This technique is also used in the Brahmaputra water bodies to trace the genetic population of Hilsa*i.e.* whether Hilsa catch from different areas are from same genetic stock. The RAPD fragments observed in the 72 individuals showed polymorphism within and between the populations. The population specific bands could not be discerned from the fragment patterns generated. The cluster diagram delineated the four different populations into two major clusters, which could be due to different spawning grounds of Hilsa. The pixel intensity for each population also shows a polymorphic relationship among each other. The RAPD analyses revels that the Hilsa populations from different regions show polymorphism with each others.

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