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## Hilsa (*Tenualosa ilisha*) Genetic Diversity and Conservation Strategies for Sustainable Wetland Management in Northeastern Bangladesh

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## ABSTRACT

An investigation was conducted on the genetic diversity of the hilsa fish in the major wetlands of Sylhet, with the objective of determining its linkage to populations from effectively maintained sanctuary regions. Field surveys and laboratory analyses, encompassing DNA extraction, PCR amplification, and cytochrome b sequencing, demonstrated genetic consistency among groups. Pairwise genetic differentiation (FST) values (0.000 to 0.017) demonstrated significant gene flow, corroborating the idea of a singular genetic pool. This genetic coherence highlights the feasibility of implementing established conservation techniques, including seasonal fishing prohibitions and the creation of sanctuaries, in Sylhet. The results underscore the significance of genetic knowledge in sustainable fisheries management and biodiversity preservation. By implementing these measures, the Sylhet region can augment hilsa production, bolster ecological services, and elevate the livelihoods of local populations. This study establishes a framework for integrating Sylhet into the national fisheries narrative, in accordance with global conservation objectives.

## INTRODUCTION

Indexed in Scopus

Hilsa (*Tenualosa ilisha*) is a fish species of considerable economic and ecological importance in Bangladesh, esteemed as a cultural symbol and a crucial component of the country's fisheries sector (**Sarker** *et al.*, **2021; Sunny** *et al.*, **2021a**). Hilsa constitutes over 12% of total fish production of Bangladesh, yielding over \$2 billion annually and directly sustaining the livelihoods of over two million fishermen, along with numerous others in related sectors (**Rahman** *et al.*, **2021; Sultana** *et al.*, **2022**). The ecological significance of hilsa is vital; as a migratory species, it promotes nutrient cycling in

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various aquatic environments and acts as a crucial component in food webs that support numerous aquatic organisms (Ali *et al.*, 2022). Moreover, hilsa possesses significant cultural and culinary importance, solidifying its role as a vital component of Bangladesh's legacy (Alok *et al.*, 2018; Sarker *et al.*, 2020).

Despite hilsa's significance, the Sylhet region, rich in wetlands such as Hakaluki Haor, Tanguar Haor, and the Surma River, remains underexploited for hilsa fisheries output (Ahammad *et al.*, 2020; Sunny *et al.*, 2020). These environmentally diversified wetlands provide optimal environments for hilsa reproduction and migration (Khan *et al.*, 2018). However, overfishing, habitat degradation, and the lack of focused management measures have impeded the progress of Sylhet's fisheries (Islam *et al.*, 2016; Barman *et al.*, 2020). Unlike Chandpur and Patuakhali, where sanctuary systems are well taken care of, Sylhet doesn't have well-coordinated conservation efforts (Islam *et al.*, 2018a). This means that its fisheries resources aren't being used efficiently, and it's not contributing as much to national hilsa production (Hasan *et al.*, 2020).

Genetic diversity is essential for fisheries management, especially for migratory species such as hilsa. Elevated genetic variety guarantees a population's flexibility to environmental alterations and bolsters its resilience against ecological stresses (Islam *et al.*, 2018b; Kuddus *et al.*, 2022). Furthermore, genetic connection among populations promotes the transfer of adaptive traits, enhancing the species' overall fitness (Barman *et al.*, 2020). Genetic studies in Bangladesh have shown that hilsa populations in different environments share a lot of genes. This makes it easier to carry out consistent conservation efforts, such as fishing bans and sanctuaries (Islam *et al.*, 2018b; Chakma *et al.*, 2022). These findings underscore the feasibility of implementing such strategies to efficiently manage the Sylhet hilsa community.

This study corresponds with national and international conservation objectives, notably Bangladesh's "Hilsa Fisheries Development Plan, which seeks to augment hilsa output by 50% by 2030 through the implementation of scientifically informed management strategies (**Chakma** *et al.*, **2022; Department of Fisheries, 2023**). This work contributes to Sustainable Development Goal 14 (Life Below Water), which underscores the sustainable utilization and preservation of marine and freshwater ecosystems on a global scale. This project aimed to incorporate Sylhet's hilsa fisheries into bigger frameworks to improve regional production, preserve biodiversity, and bolster the socioeconomic welfare of residents.

The main aim of this project was to utilize genetic insights to develop and execute sustainable management methods for hilsa in Sylhet. The goals were: (1) to look at the genetic diversity and population structure of hilsa in the wetlands of Sylhet; (2) to find out how genetically connected the hilsa populations in Sylhet are to those in wellmanaged sanctuary areas, and (3) to suggest specific management strategies, like

stopping fishing during certain times of the year, setting up sanctuaries, and communityled conservation efforts, to increase hilsa production and to protect biodiversity in Sylhet.

### **MATERIALS AND METHODS**

### 1. Data acquisition and sampling

The research was carried out over 12 months, from August 1, 2022 to June 20, 2023. This study encompassed both fieldwork and laboratory activities. Both qualitative and quantitative empirical data were collected through household surveys, field observations, focus group discussions (FGDs), oral histories, and interviews with key informants (KII).

### 2. Questionnaire-based interviews

This study involved individual interviews with resource users gathered data on fish genetic resources, aquatic biodiversity, and hilsa management issue. The interviews included multiple-choice questions featuring both quantitative and qualitative elements, alongside open-ended and predetermined inquiries. A total of 90 exploratory interviews using a semi-structured questionnaire were conducted. Each interview lasted approximately 60 to 70 minutes. In addition, a total of 20 focus group discussions were conducted, each with 7 to 8 participants. Furthermore, 10 key informant interviews (KII) with local entrepreneurs and NGO representatives to validate and enhance the data were also conducted. Participants provided views on sustainability, dangers and conservation measures.

### **3. Sampling of fish species**

Field surveys and sampling within local fishing communities, which included fish markets and landing sites adjacent to the designated wetlands, were conducted. Moreover, quarterly sampling at landing centers and marketplaces near the wetlands were conducted to record the diversity of fish species and their seasonal variations. Furthermore, taxonomic identification by cross-referencing the Catalogue of Life 2017 annual checklist with the IUCN Red List of Threatened Species (Version 2017-1) were conducted. This method guaranteed precise categorization of the taxa and their conservation status.

## 4. Analysis of morphometric characteristics

The morphometric parameters of *Tenualosa ilisha* were measured with great precision to enable precise morphological analysis. Measurements including total length (TL), fork length (FL), standard length (SL), caudal peduncle length (CpL), head length

(HL), eye diameter (ED), snout length (SnL), and post orbital length (POL) were documented.

### 5. Analysis of meristic characteristics

The meristic characteristics of *T. ilisha* were analyzed to enhance taxonomy identification and population differentiation. Traits including dorsal fin rays (D), pectoral fin rays (P1), pelvic fin rays (P2), anal fin rays (A), lateral line scales (L.l), and branchiostegals (Br) were enumerated with a magnifying glass to ensure precision.

## 6. Compilation of hilsa samples

Specimens of *Tenualosa ilisha* were obtained from three principal sites in Sylhet: Hakaluki Haor, Tanguar Haor, and the Surma River. A total of sixty fish samples were collected, averaging 250 grams in weight, from each location. The samples were promptly kept in ice and sent them to the Department of Genetic Engineering and Biotechnology at Shahjalal University of Science and Technology. Dorsal fins were surgically removed in the laboratory and stored in 100% ethanol for long-term preservation at -20°C.

### 7. Extraction of DNA

The GeneJET DNA Extraction Kit was used to isolate mitochondrial DNA (mtDNA) from muscle tissue, following the manufacturer's instructions. The isolated DNA were preserved at -20°C to prepare it for future investigations. This technique guaranteed high-quality DNA appropriate for subsequent uses.

### 8. Polymerase chain reaction amplification

The mtDNA cytochrome b regions were amplified using the polymerase chain reaction (PCR) method. Table (1) specifies the primers used in this procedure. There were 5µl of 1X buffer, 2.5mM of each dNTP, 5 pmoles of each primer, 1.5mM MgCl<sub>2</sub>, 3 units of Taq polymerase (Genei, India), and 25 to 50ng of genomic DNA in each 50µl mixed PCR reaction. The PCR technique comprised an initial denaturation at 94°C for five minutes, succeeded by 29 cycles of denaturation at 94°C for 30 seconds, annealing at 55°C for one minute, and extension at 72°C for 90 seconds, culminating in a final extension at 72°C for 10 minutes. Gel electrophoresis was employed on a 0.8% agarose gel to assess the quality of the amplified DNA, and then recorded it using a gel documentation system.

## 9. Sequencing of the cytochrome b region

An applied biosystems 3130 genetic analyzer was used to sequence the purified PCR products for the cytochrome b region of *T. ilisha*'s mitochondrial DNA. For every

 $20\mu$ l sequencing reaction, there was 10ng of purified template DNA,  $4.0\mu$ l of the readymade reaction premix,  $2.0\mu$ l of big dye terminator buffer, and  $0.32\mu$ l of primer. The sequences were examined for genetic variation and were uploaded to GenBank for future reference.

## 10. Data analysis

The BioEdit sequence alignment editor was used to align and trim the chromatographs of the sequenced data. Then the sequences were uploaded to GenBank and BLAST was used (www.ncbi.nlm.nih.gov) to identify homologous sequences. Genetic variation among populations was assessed using DnaSP v6.0 software. Phylogenetic relationships were deducted using the neighbor-joining method, employing the Kimura 2-parameter model, with 1000 bootstrap replications to ensure robustness. Evolutionary distances, quantified as base substitutions per site, were calculated using MEGA11. Pairwise genetic differentiation (FST) values were computed to assess population structure and gene flow, providing insights into the genetic connectivity of *T. ilisha* populations.

## **RESULTS AND DISCUSSION**

## 1. Morphological and meristic analyses

A total of 90 hilsha samples were collected, with 30 samples collected from 3 ecologically significant regions in the Sylhet belt, namely Hakaluki Haor, Tanguar Haor, and the Surma River. These areas represent vital inland freshwater habitats that support diverse aquatic ecosystems and serve as key breeding and migration zones for hilsha. 30 samples were obtained from each location to ensure representative coverage of the population in this region.

The samples were examined in detail to assess their morphometric and meristic characteristics. Morphometric traits, including total length (TL), fork length (FL), standard length (SL), caudal peduncle length (CpL), head length (HL), and eye diameter (ED), among others, were measured. Proportional ratios such as TL:SL, TL:FL, and HL:SnL were calculated to ensure precise identification. Additionally, meristic counts, including dorsal fin rays, pectoral fin rays, pelvic fin rays, and lateral line scales, were recorded. The collected data are summarized in Tables (1, 2). The morphological data were scaled to the observed mean total length of  $24 \pm 1$ cm in the Sylhet belt population. These measurements were then compared with previously reported values for freshwater Hilsha populations, showing a high degree of consistency.

The Sylhet belt samples exhibited morphometric characteristics that align closely with the established ranges for freshwater *Tenualosa ilisha*. Table (1) summarizes the comparative data.

| Characteristic               | Sylhet belt hilsha Mean ± SD (cm) |  |  |  |
|------------------------------|-----------------------------------|--|--|--|
| Total length (TL)            | $24.00 \pm 1.00$                  |  |  |  |
| Fork length (FL)             | $20.88 \pm 0.26$                  |  |  |  |
| Standard length (SL)         | $18.96\pm0.12$                    |  |  |  |
| Caudal peduncle length (CpL) | $1.51\pm0.08$                     |  |  |  |
| Head length (HL)             | $5.76\pm0.09$                     |  |  |  |
| Eye diameter (ED)            | $0.96\pm0.03$                     |  |  |  |
| Snout length (SnL)           | $1.34\pm0.04$                     |  |  |  |
| Post orbital length          | $3.63\pm0.11$                     |  |  |  |

| Table 1. Morphometric characteristics of | f Sylhet belt hilsha |
|--|----------------------|
|--|----------------------|

Key proportional ratios derived from Sylhet belt hilsha also closely matched those documented for freshwater hilsha populations, as shown in Table (2).

**Table 2.** Proportional ratios of Sylhet belt hilsha

| Proportional ratios | Sylhet belt hilsha ratios |  |  |  |
|---------------------|---------------------------|--|--|--|
| TL:SL               | $1.27\pm0.005$            |  |  |  |
| TL:FL               | $1.15 \pm 0.01$           |  |  |  |
| SL:FL               | $0.91\pm0.009$            |  |  |  |
| TL:CpL              | $15.92 \pm 0.80$          |  |  |  |
| SL:CpL              | $12.50 \pm 0.65$          |  |  |  |
| HL:SnL              | $4.30\pm0.60$             |  |  |  |
| HL:Post orbital     | $1.59\pm0.08$             |  |  |  |

The meristic traits of Sylhet belt hilsha, including fin ray counts and lateral line scale numbers, also fell within the documented ranges for freshwater hilsha populations, as presented in Table (3).

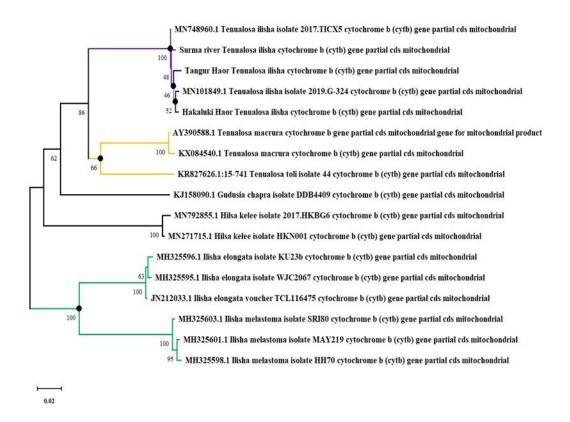
| Meristic characteristic   | Sylhet belt hilsha |  |  |
|---------------------------|--------------------|--|--|
| Dorsal fin rays (D)       | 18                 |  |  |
| Pectoral fin rays (P1)    | 14                 |  |  |
| Pelvic fin rays (P2)      | 8                  |  |  |
| Anal fin rays (A)         | 19                 |  |  |
| Lateral line scales (L.l) | 47                 |  |  |
| Branchiostegals (Br)      | VI                 |  |  |

## **Table 3.** Meristic characteristics of Sylhet belt hilsha

The morphological and meristic data, as well as the proportional ratios, demonstrated a strong alignment with the established characteristics of freshwater *Tenualosa ilisha*. These results confirm, beyond any doubt, that the Sylhet belt samples belong to the hilsha fish species. This morphological uniformity provides a reliable basis for further genetic analyses, as discussed in subsequent sections.

## 2. Phylogenetic analysis

The genetic relationship among T. ilisha populations from Hakaluki Haor, Tanguar Haor, and the Surma River was elucidated through a neighbor-joining phylogenetic tree, constructed using the ClustalW alignment algorithm and the Kimura 2-parameter (K2P) distance model (Fig. 1). The analysis provided a robust framework for evaluating the evolutionary relationships within and between populations in this region. All samples from the three habitats were clustered into a single clade, with exceptionally high bootstrap support values (reaching 100%). This clustering conclusively demonstrates a close genetic relationship among the populations and provides strong evidence for their classification as a single genetic group. Minimal evolutionary distances were observed between populations, further supporting the hypothesis of high genetic similarity. These results suggest that gene flow among these populations is substantial, likely facilitated by their shared geographic distribution and interconnected aquatic systems. The analysis also highlighted the genetic proximity of Sylhet-region T. ilisha to related species, such as Gudusia chapra, Tenualosa macrura, and Tenualosa toli, while distinguishing them from more distantly related species, including *Ilisha melastoma* and *Hilsa kelee*. This broader phylogenetic insight underscores the unique genetic cohesion of the T. ilisha populations in this specific region.



**Fig. 1.** The evolutionary history inferred by the neighbor-joining method and the optimal phylogenetic tree illustrated. The proportion of occurrences of the related taxa clustered in the bootstrap test (with 1000 repetitions) is displayed beneath the branches. The evolutionary distances were computed with the Kimura 2-parameter approach, shown as the number of base substitutions per site

## 3. Genetic diversity

The genetic diversity of *T. ilisha* populations from Hakaluki Haor, Tanguar Haor, and the Surma River was assessed using 744 nucleotide sequences. The analysis identified three distinct haplotypes among the populations, with 271 segregating (polymorphic) sites observed. The average nucleotide frequencies across all populations were A = 28%, T = 24%, C = 30%, and G = 18%, with an average %G~C content of 47.73%. These values reflect a balanced nucleotide composition, characteristic of populations with a conserved genetic structure.

Haplotype diversity (Hd) was calculated as 1.000, indicating that all samples represented unique haplotypes, which points to a highly diverse population despite their geographical proximity. Nucleotide diversity (PiJC) was 2.50198, further supporting moderate genetic variation essential for the adaptability and long-term resilience of these populations.

Pairwise genetic differentiation was quantified using the fixation index (FST), a measure that evaluates the extent of genetic variation among populations. The FST values within Sylhet populations (Hakaluki Haor, Tanguar Haor, and Surma River) ranged from 0.000 to 0.011, signifying negligible differentiation. For example: Between Hakaluki Haor and Surma River: FST = 0.003, indicating minimal genetic difference; between Hakaluki Haor and Tanguar Haor: FST = 0.011, suggesting slightly higher differentiation but still reflecting genetic uniformity. These low FST values strongly suggest a high level of gene flow between these populations, facilitated by interconnected aquatic systems within the Sylhet region. Such gene flow homogenizes genetic diversity and supports the hypothesis of a single genetic stock for *T. ilisha* in the Sylhet region.

The genetic relationships between Sylhet populations and those from other regions were also examined. Pairwise FST values comparing Sylhet populations with sequences from India and other parts of Bangladesh (e.g., MN101849.1, MN748955.1) ranged from 0.000 to 0.017. While these values are slightly higher than those observed within Sylhet populations, they still suggest moderate genetic similarity between Sylhet *T. ilisha* and external populations. For example, the FST between Hakaluki Haor and an Indian sequence (MN101849.1) was 0.005, indicating low differentiation, while the FST between Tanguar Haor and another Bangladeshi sample (MN748960.1) was 0.003, suggesting very low differentiation.

The shared nucleotide frequencies, haplotypes, and low FST values collectively confirm that *T. ilisha* populations in Sylhet exhibit genetic uniformity.

| Population pairs       | FST value | Interpretation                                    |  |  |  |
|------------------------|-----------|---|--|--|--|
| Hakaluki Haor vs.      | 0.003     | Indicates negligible differentiation; populations |  |  |  |
| Surma River            |           | are genetically similar.                          |  |  |  |
| Hakaluki Haor vs.      | 0.011     | Slightly higher value, but still reflects minimal |  |  |  |
| Tanguar Haor           |           | differentiation.                                  |  |  |  |
| Surma River vs.        | 0.011     | Similar to Hakaluki Haor vs. Tanguar Haor,        |  |  |  |
| Tanguar Haor           |           | suggesting uniformity.                            |  |  |  |
| Sylhet populations vs. | ~0.005-   | Indicates moderate genetic similarity but         |  |  |  |
| India                  | 0.017     | slightly higher differentiation.                  |  |  |  |

**Table 4.** Pairwise FST values among populations

## 4. Single nucleotide polymorphisms and deletions

The identification of single nucleotide polymorphisms (SNPs) and deletions provides valuable insights into the genetic variability and evolutionary dynamics of the

*Tenualosa ilisha* populations in Hakaluki Haor, Tanguar Haor, and Surma River. A total of two SNPs were identified in the Hakaluki Haor sequence, namely A4G and A13T. These findings indicate a relatively stable genetic environment with minimal mutation pressure in this population.

In contrast, the Tanguar Haor population demonstrated a markedly higher degree of genetic variability, with seven SNPs detected across its partial sequence: A6G, C10G, A24G, C29A, A104T, C165A, and T738G. The abundance of SNPs in this population suggests the influence of increased genetic pressure, which may be attributed to environmental variability, habitat-specific selective pressures, or genetic drift. Such genetic variation could play a pivotal role in facilitating local adaptation to ecological niches within the haor ecosystem.

Interestingly, the Surma River population exhibited a distinct pattern of genetic differentiation characterized by two nucleotide deletions. These deletions, both thymine bases, were identified at positions 611 and 736 in the sequence. While deletions are less common than SNPs, they may have functional implications on gene expression or protein structure, potentially contributing to unique phenotypic or adaptive traits in this population.

## 5. Conserved sequence analysis

The alignment of nucleotide sequences of *Tenualosa ilisha* from Hakaluki Haor, Tanguar Haor, and the Surma River, generated using the ClustalW algorithm and visualized with CLC Sequence Viewer. This conserved sequences were built by incorporating nucleotides shared identically across all three populations, while partial conservation included nucleotides common to only two populations. Loci with complete variability were marked as "N" to denote genetic inconsistency.

In the alignment, conserved regions demonstrated strong genetic similarity, supporting the hypothesis of a unified genetic stock for *T. ilisha* in the Sylhet region (Fig. 2). Variability at specific loci, marked by "N," highlights genetic divergence likely influenced by environmental factors or evolutionary adaptations. Visual representation in Fig. (5) emphasizes that the majority of nucleotide positions were conserved, underscoring genetic uniformity among the populations.

|                                   |                          |                          | 8                        |                                     | 8                                       |                          |            |
|-----------------------------------|--------------------------|--------------------------|--------------------------|-------------------------------------|---|--------------------------|------------|
| Hakaluki<br>Tangur                | CGGACGTGAA               | GTACTCTGGG               |                          | 40<br>I<br>GGTTGGGTGA<br>GCTTGGGTGA | GAAAAGGGCT<br>GAAAA <mark>A</mark> GGCT | AGGGATGTCA<br>CGGGATGTCA |            |
| Surma                             | CGGACGTGAA               |                          | CTAGTA                   | GGTTGGGTGA                          |   | AGGGATGTCA               |            |
| 100%<br>Conservation              |                          |                          |                          | GNTTGGGTGA                          | GAAAANGGCT                              | ndddardrea               |            |
| 0%                                |                          | 80                       |                          | 100                                 |   | 120                      |            |
|                                   | GGGCCAGGAG<br>GGGCCAGGAG | CATAACTGCG<br>CATAACTGCG |                          | GGTCTTTGTA                          | AGAAAAGTAT                              | GGGTGGAATG<br>GGGTGGAAAG | 120<br>105 |
| Surma<br>Consensus                |                          | TATAACTGCG<br>NATAACTGCG | AAGCCCAGCA<br>AAGCCCAGCA | GGTCTTTGTA<br>GGTCTTTGTA            | AGAAAAGTAT<br>AGAAAAGTAT                | GGGTGGAATG               | 96         |
| Conservation                      |                          |                          |                          |                                     |   |                          |            |
| 0%                                |                          | 140                      |                          | 160                                 |   | 180                      |            |
|                                   | AAATTTTATC<br>AAATTTTATC | AGCGTCTGAG               | TTTAGCCCCG<br>TTTAGCCCCG | CAGGGTTGTT                          | AGATCCTGTC<br>AGATCCTGTC                | TCGTGTAGAA<br>TCGTGTAGAC | 180<br>165 |
|                                   | AAATTTTATC               | AGCGTCTGAG<br>AGCGTCTGAG | TTTAGCCCCG               |                                     | AGATCCTGTC<br>AGATCCTGTC                | TCGTGTAGAA<br>TCGTGTAGAN | 156        |
| 100%<br>Conservation              |                          |                          |                          |                                     |   |                          |            |
| 0%                                |                          | 200                      |                          | 220<br>1                            |   | 240<br>I                 |            |
|                                   | ATAAGAGGTG<br>ATAAGAGGTG |                          | GCTCCTGCGA<br>GCTCCTGCGA | TTACGAATGG<br>TTACGAATGG            |   | TGGAAGGCGA<br>TGGAAGGCGA |            |
|                                   | ATAAGAGGTG<br>ATAAGAGGTG | TAGGATGGTG<br>TAGGATGGTG | GCTCCTGCGA<br>GCTCCTGCGA |                                     | GAACAAGAAG<br>GAACAAGAAG                | TGGAAGGCGA<br>TGGAAGGCGA | 216        |
| 100%<br>Conservation              |                          |                          |                          |                                     |   |                          |            |
| 0%                                |                          | 260<br>                  |                          | 280<br>1                            |   | 300<br>1                 |            |
| Tangur                            | AAAATCGAGT               | GAGGGTGGCG<br>GAGGGTGGCG | TTATCTACGG<br>TTATCTACGG | AAAAACCGCC                          | TCAAATTCAT<br>TCAAATTCAT                | TGAACGAGCT<br>TGAACGAGCT |            |
| Surma<br>Consensus                | AAAATCGAGT<br>AAAATCGAGT | GAGGGTGGCG<br>GAGGGTGGCG | TTATCTACGG<br>TTATCTACGG | AAAAACCGCC<br>AAAAACCGCC            | TCAAATTCAT<br>TCAAATTCAT                | TGAACGAGCT<br>TGAACGAGCT | 276        |
| Conservation                      |                          |                          |                          |                                     |   |                          |            |
|                                   |                          | 320<br>I                 |                          | 340<br>1                            |   | 360<br>I                 |            |
| Tangur                            |                          | GTAAGGTACG<br>GTAAGGTACG | GCAGATAGCA               | GGTTTGTAAT                          | AACTGTAGCG                              | CCTCAGAAGG<br>CCTCAGAAGG | 345        |
| Consensus                         | CGTTCCCTAC<br>CGTTCCCTAC | GTAAGGTACG               | GCAGATAGCA<br>GCAGATAGCA | GGTTTGTAAT                          | AACTGTAGCG                              | CCTCAGAAGG<br>CCTCAGAAGG | 336        |
| Conservation                      |                          |                          |                          |                                     |   |                          |            |
| Hakaluki                          | ACATTTGTCC               | CCAAGGAAGG               | ACATAGCCAA               |                                     | CATTATGACT                              | 420<br>I<br>AGTAATAACA   | 420        |
| Tangur                            | ACATTTGTCC               | CCAAGGAAGG               | ACATAGCCAA               | CGAAGGCCGT                          | CATTATGACT                              | AGTAATAACA               | 405        |
|                                   | ANATTTGTCC               | CCAAGGAAGG               |                          | CGAAGGCCGT                          | CATTATGACT                              | AGTAATAACA               | 000        |
| Conservation                      |                          |                          |                          |                                     |   |                          |            |
| Hakaluki                          | GGATAACTCC               | GATGTTTCAG               | GTTTCTTTGT               | ATAGGTAGGA                          | GCCATAGTAG                              | AGTCCTCGGC               | 480        |
| Tangur<br>Surma                   | GGATAACTCC<br>GGATAACTCC | GATGTTTCAG<br>GATGTTTCAG | GTTTCTTTGT<br>GTTTCTTTGT | ATAGGTAGGA<br>ATAGGTAGGA            | GCCATAGTAG<br>GCCATAGTAG                | AGTCCTCGGC<br>AGTCCTCGGC |            |
| Consensus                         | GGATAACTCC               | GATGTTTCAG               | GTTTCTTTGT               | ATAGGTAGGA                          | GCCATAGTAG                              | AGTCCTCGGC               |            |
| Conservation                      |                          | 500                      |                          | 520                                 |   | 540                      |            |
|                                   |                          | GTAGATGCAC               |                          |                                     |   | ACATTACGGA               |            |
| Surma                             | CGATATGGGC               |                          | ATGAAGAAAA               | AGGATGCTCC                          | GTTTGCGTGC                              | ACATTACGGA               |            |
| Consensus<br>100%<br>Conservation | CGATATGGGC               | GTAGATGCAC               | ATGAAGAAAA               | AGGATGCTCC                          | GTTTGCGTGC                              | ACATTACGGA               |            |
| 0%                                |                          | 560                      |                          | 580                                 |   | 600                      |            |
|                                   |                          | GTAATTGACG<br>GTAATTGACG |                          |                                     |   | GCGGTTGCGA               |            |
|                                   |                          | GTAATTGACG<br>GTAATTGACG |                          | TGTGTGTAAC<br>TGTGTGTAAC            | TGATGAAAAT<br>TGATGAAAAT                | GCGGTTGCGA<br>GCGGTTGCGA |            |
| Conservation                      | TEAGTEACCE               | GTAATTGACG               | TCACGGCAGA               | TGTGTGTAAC                          | 19419AAAA                               | GCGGTTGCGA               |            |
| 0%                                |                          | 620<br>1                 |                          | 640<br>1                            |   | 660<br>1                 |            |
| Tangur                            | TATCAGAGGT               | GTAGTGCATA<br>GTAGTGCATA | GCCAGGAAGA               | GTCCTGTCAA                          | GATTTGTGAT                              | GCCAAACAAA               | 645        |
| Surma<br>Consensus                | TATCAGAGGT               | GTAGTGCATA<br>GTAGTGCATA | GCCAGGAAGA               | GTCC-GTCAA                          | GATTTGTGAT<br>GATTTGTGAT                | GCCAAACAAA               | 635        |
| Conservation                      |                          |                          |                          |                                     |   |                          |            |
|                                   | 00007.000                | 680<br>1                 |                          | 700                                 |   | 720                      | 70-        |
| Tangur                            | GCCCTAGGAG               | AGATCCGAAG               | TTTCATCAGA               | CCGAGATATT                          | GGAGGGAGCT<br>GGAGGGAGCT                | GGGAGATCAA               | 705        |
|                                   |                          | AGATCCGAAG<br>AGATCCGAAG |                          |                                     |   |                          | 092        |
| Conservation<br>0%                |                          |                          |                          |                                     |   |                          |            |
| Hakaluki                          | CTACTGCGTC               |                          | TTTA                     | 760<br>1                            | 744                                     |                          |            |
| Tangur                            | CTACTGCGTC               | GTTAGCGATT               | TTTAGGAGTG               | GTTGGCTCT-<br>GGTGGGTTTT            | 744                                     |                          |            |
|                                   | CTACTGCGTC               | GTTAGCGATT               |                          | GNTGGNTNTT                          |   |                          |            |
| Conservation                      |                          |                          |                          | <u>Dollanonan</u>                   |   |                          |            |
|                                   |                          |                          |                          |                                     |   |                          |            |

Fig. 2. Alignment of nucleotide sequences of *Tenualosa ilisha* collected from northeastern Bangladesh

### 4. Genetic insights and implications for management

The genetic uniformity observed among hilsa populations in Sylhet's aquatic ecosystems (Hakaluki Haor, Tanguar Haor, and Surma River) underscores a significant finding, the Sylhet Hilsa populations are genetically indistinguishable from those in well-managed sanctuary areas such as Patuakhali, Chandpur, and Cox's Bazar. Fixation index (FST) values ranging from 0.000 to 0.011 suggest negligible genetic differentiation, confirming substantial gene flow facilitated by interconnected water systems. These results align with findings from similar studies, which have demonstrated the role of shared aquatic habitats in maintaining genetic connectivity among migratory fish species (**Barman** *et al.*, 2020; Rahman *et al.*, 2021).

Such genetic uniformity has practical implications for management. The presence of a single genetic stock means that proven strategies employed in other hilsa-rich regions can be effectively adapted to Sylhet. For instance, the successful application of seasonal fishing bans and sanctuary establishment in the Meghna and Padma river systems has led to significant increases in hilsa production and population recovery (**Islam** *et al.*, **2011**). The implementation of similar strategies in Sylhet can thus yield comparable benefits, ensuring both ecological and economic sustainability.

These findings align with other genetic analyses, confirming the high degree of genetic connectivity among the populations, which are part of a shared aquatic ecosystem. This analysis strengthens the case for treating these populations as a single genetic unit, essential for their effective conservation and management.

## 4.1 Integration of morphological and genetic findings

The morphological and meristic characteristics of Sylhet hilsa further corroborate the genetic findings. Consistency in traits such as total length, fork length, and fin ray counts with documented freshwater hilsa populations highlights their taxonomic alignment (**Siddique** *et al.*, **2019**). This congruence supports the hypothesis that Sylhet's hilsa fish are ecologically and genetically part of the broader migratory population, reinforcing the need for uniform management policies.

## 4.2 Proposed management strategies for Sylhet

## 4.2.1 Seasonal fishing bans

The introduction of seasonal fishing bans during critical breeding periods is a cornerstone of sustainable fisheries management. Evidence from Bangladesh's sanctuary areas shows that targeted bans during peak spawning times have significantly enhanced recruitment and replenishment of hilsa populations (Ahmed *et al.*, 2020; Sunny *et al.*,

**2021b**). Implementing similar bans in Sylhet, synchronized with the national breeding calendar, can prevent overfishing and ensure long-term population viability.

### 4.2.2 Establishment of Hilsa sanctuaries

Designating key habitats such as Hakaluki Haor and Tanguar Haor as hilsa sanctuaries can provide critical safe zones for breeding and juvenile growth. These wetlands offer ideal ecological conditions, including ample food resources and shelter for hilsa at various life stages (Islam & Chuenpagdee, 2017). Studies from the Padma River sanctuaries have shown that such measures lead to a 30% increase in juvenile survival rates (Khatun *et al.*, 2021). Establishing sanctuaries in Sylhet will likely yield similar ecological benefits while promoting biodiversity conservation.

## 4.2.3 Community engagement and compliance

Engaging local communities in conservation efforts is essential for the success of any management strategy (**Islam & Chuenpagdee, 2018**). Fishermen's compliance with fishing bans and sanctuary regulations is directly linked to their socioeconomic incentives (**Hasan** *et al.*, **2020**). Involving community leaders in decision-making and providing alternative livelihoods during fishing bans, such as aquaculture training or ecotourism development, can enhance community support and reduce enforcement challenges. Examples from Chandpur's sanctuary programs demonstrate that active community participation reduces illegal fishing activities by 40% (**Siddique et al., 2019**).

### 4.2.4 Scientific monitoring and policy adaptation

Continuous scientific monitoring of genetic diversity, population dynamics, and ecological health is critical for adaptive management. The integration of molecular tools, such as DNA barcoding and SNP analysis, can provide real-time insights into population structure and gene flow. Regular assessments will enable policymakers to fine-tune strategies based on empirical evidence, ensuring their long-term efficacy.

### 4.2.5 Contributions to biodiversity conservation and livelihood development

The establishment of a sustainable Hilsa fishery in Sylhet has dual benefits for biodiversity conservation and local livelihoods. By maintaining the genetic integrity and population size of Hilsa, conservation efforts can support the broader wetland ecosystem. Hilsa act as a keystone species, contributing to the ecological stability of aquatic habitats. Their conservation indirectly supports other species dependent on the same ecosystem, enhancing overall biodiversity (**Ali** *et al.*, **2022; Sunny** *et al.*, **2023**).

On the socioeconomic front, increased Hilsa production can significantly uplift the livelihoods of local fishing communities. Studies indicate that a 20% rise in hilsa catch can result in a 15% increase in fishermen's annual income (**Rahman** *et al.*, 2020).

Moreover, alternative livelihood programs during off-seasons can provide economic resilience, reducing dependency on unsustainable fishing practices.

## 4.2.6 Relevance to national fisheries policy

Integrating Sylhet's hilsa fisheries into the national framework can strengthen Bangladesh's position as a global leader in sustainable fisheries management. The success of hilsa sanctuaries and fishing bans in other regions provides a replicable model for Sylhet, supported by robust scientific evidence. By adopting these strategies, Sylhet can contribute to the national target of achieving a 50% increase in hilsa production by 2030, as outlined in the "Hilsa Fisheries Development Plan" (**Department of Fisheries**, **2023**).

### CONCLUSION

This study indicates that the hilsa populations in Sylhet are components of a broader interconnected stock in Bangladesh, exhibiting significant genetic homogeneity. This indicates the necessity of incorporating fisheries into the national Hilsa conservation strategy. By implementing effective management strategies such as seasonal fishing prohibitions, creating hilsa sanctuaries, and engaging local people, Sylhet can improve its fisheries production and secure the long-term viability of its aquatic ecosystems. This would enhance the livelihoods of millions in Bangladesh since the hilsa fisheries sustain their economic well-being. The research underscores the significance of scientific monitoring and adaptive management in fisheries conservation, highlighting that regular genetic evaluations and ecological surveys are essential for evaluating the efficacy of management actions. The sustainable management of Sylhet's hilsa fishery can exemplify the equilibrium between biodiversity conservation and livelihood enhancement, securing enduring advantages for both humanity and the environment.

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