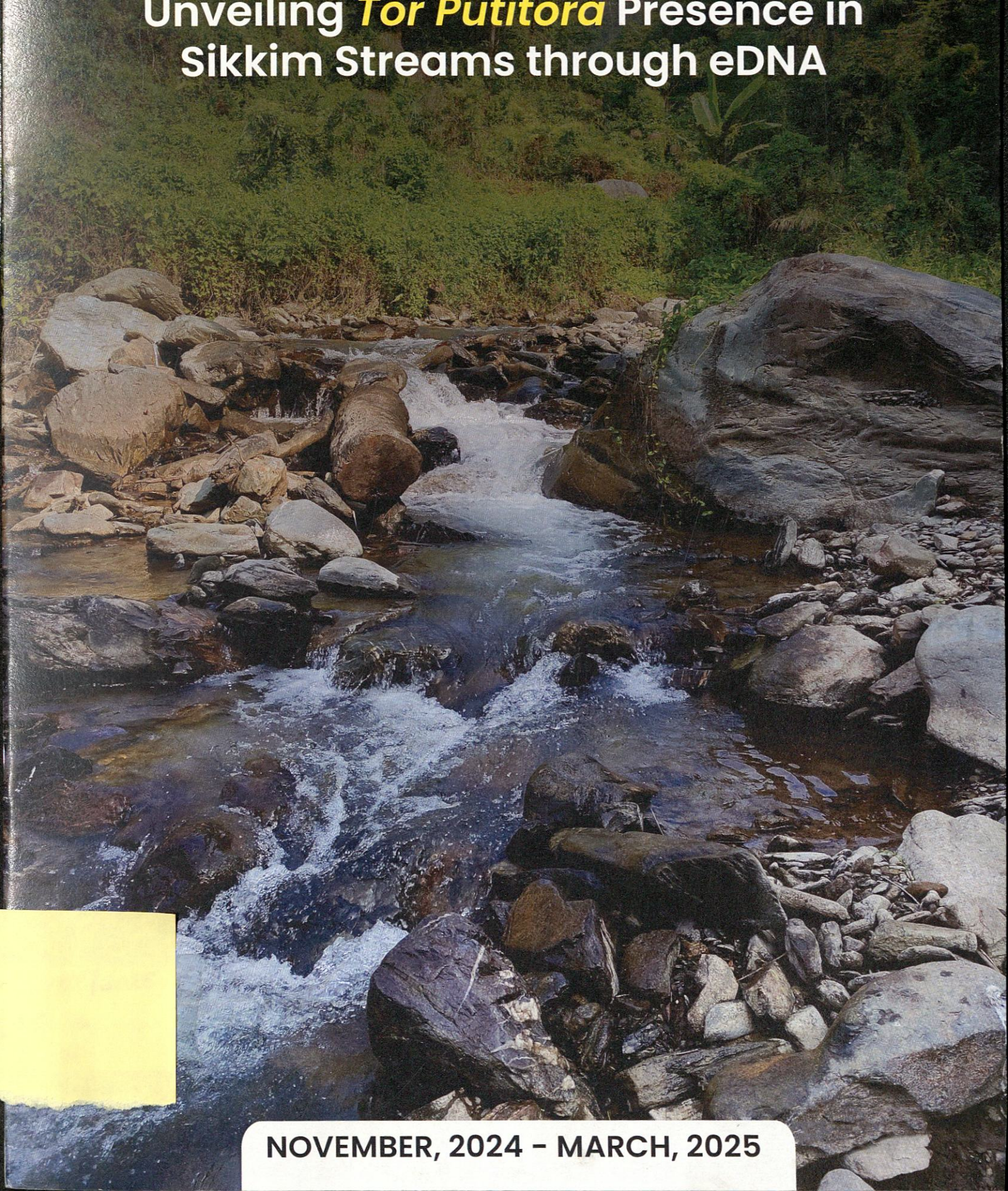
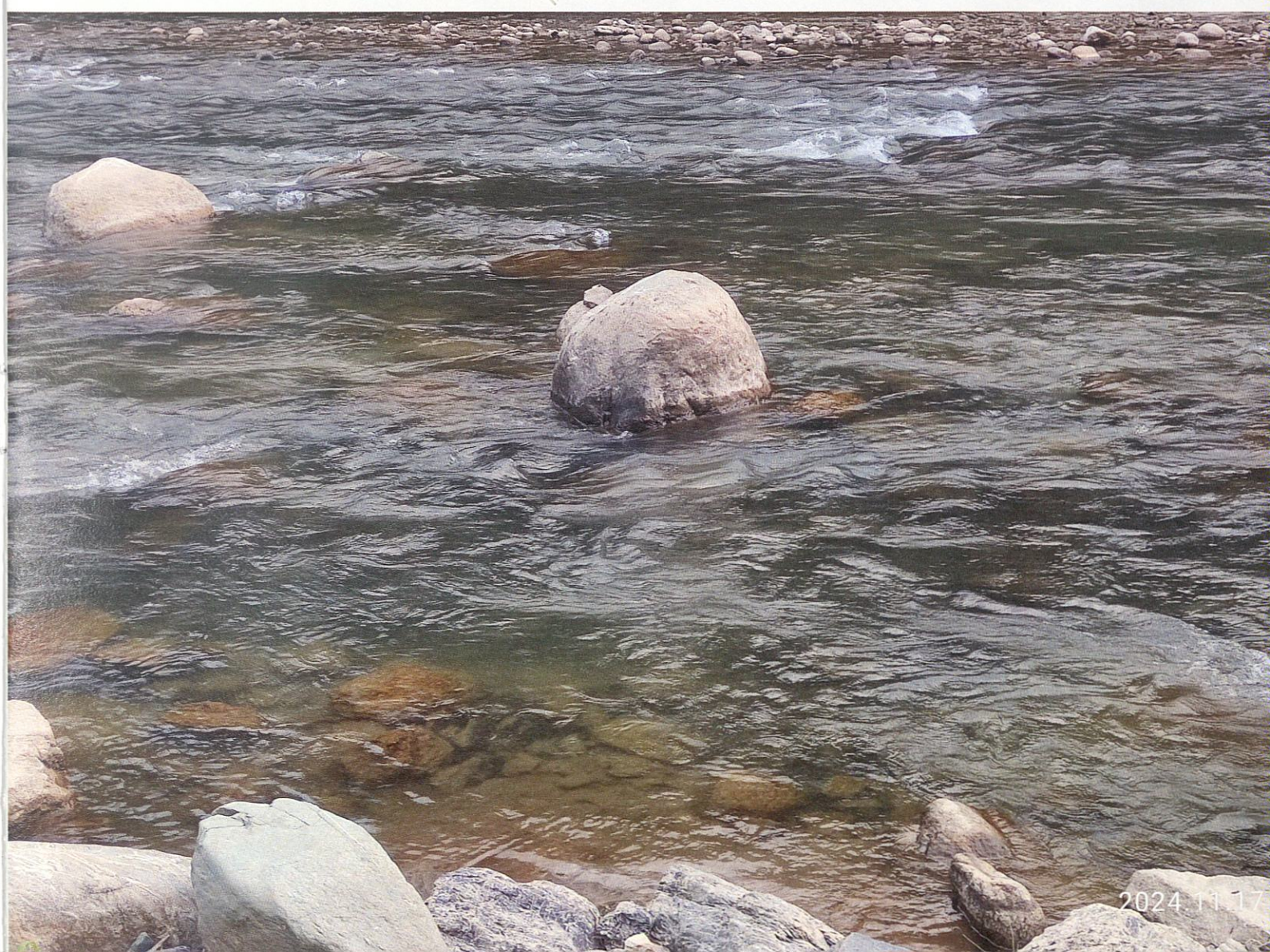
Unveiling *Tor Putitora* Presence in Sikkim Streams through eDNA



NOVEMBER, 2024 - MARCH, 2025

ABSTRACT

Environmental DNA (eDNA) metabarcoding was used to detect the presence of *Tor putitora* (Golden Mahseer) in selected streams of Sikkim. Water samples were collected from six sites based on habitat characteristics conducive to the species' survival. The collection of samples was followed by filtration of water samples, isolation of the DNA, PCR and sequencing targeting the COI region were conducted under aseptic conditions. The results confirmed *Tor putitora* presence at two locations (BR_06 and BR_08) with high certainty (>99%), aligning with previous studies on its distribution in Sikkim. These results indicate that these streams serve as habitats for *Tor putitora*. These findings highlight the potential of these streams to support mahseer populations, reinforcing the need for conservation efforts to protect these critical habitats from ecological threats like- ecological pressures, including hydropower and linear infrastructure developments. This study adds to the expanding evidence concerning mahseer distribution in Sikkim and highlights the critical need for conservation initiatives aimed at safeguarding its essential habitats, by incorporating appropriate mitigation measures. Continued research and long-term monitoring are imperative to evaluate population dynamics and ensure the sustainable management of *Tor putitora* in the region.



INTRODUCTION

eDNA metabarcoding, a highly sensitive and non-invasive method for identifying species is environmental DNA (eDNA) metabarcoding, that detects genetic material released into aquatic ecosystems. Identification of DNA samples in the river systems aids in enabling the effective monitoring of *Tor putitora* populations, even at low concentrations.

In Himalaya freshwater habitats, Golden mahseer (*Tor putitora*) are keystone species that are essential to preserving ecological balance. However, the Teesta River and its tributaries in Sikkim were known to have abundant populations of the species. The recent studies show a decline in their populations that raised the concerns for conservation (Goswami *et al.*, 2018; Baruah *et al.*, 2012).

Tor putitora populations in Sikkim are currently limited to some river segments, resulting in a fragmented distribution. *Tor putitora* populations are further threatened by the growth of hydroelectric plants, changes in river hydrodynamics, climate-changes competition from exotic fish species (Gupta *et al.*, 2020). An integrated conservation strategy that incorporates habitat restoration, stringent fisheries laws, and sustainable management techniques is required to slow their decline. In Sikkim's riverine habitats, long-term monitoring using eDNA metabarcoding will be essential for population tracking and directing conservation efforts.

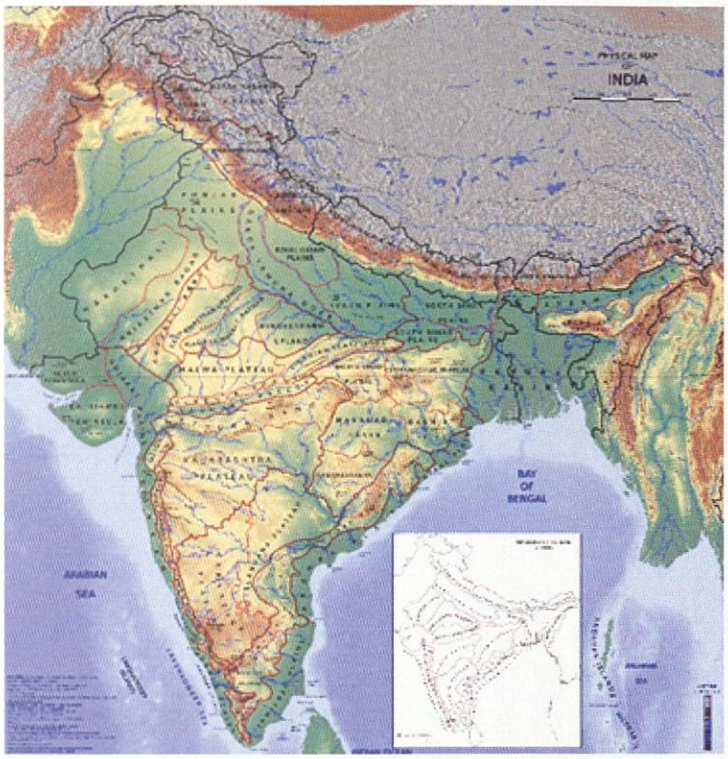
OBJECTIVES

1. To assess the potential impacts of ADB Sikkim Road Upgradation Project on the aqautic ecosystem by evaluating the presence of *Tor putitora* using environmental DNA (eDNA) metabarcoding.

2. To access the spatial distribution of *Tor putitora* across six sampling sites: BR_04, BR_06 (Ramam Bridge), BR_08 (Matizar-Kitam Bridge), BR08_S (a stream 130 meters from BR_08), T (Teesta Main Stream), and Malli.

MATERIAL & METHOD

STUDY SITE:



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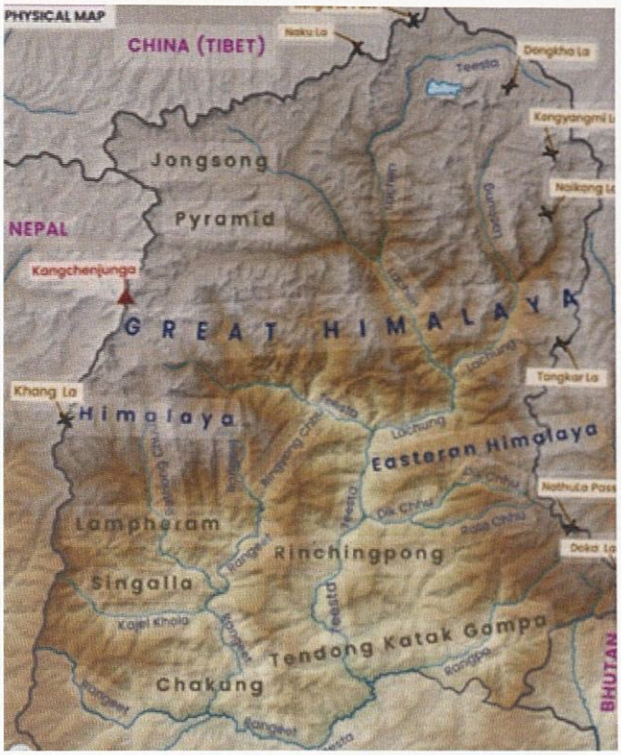

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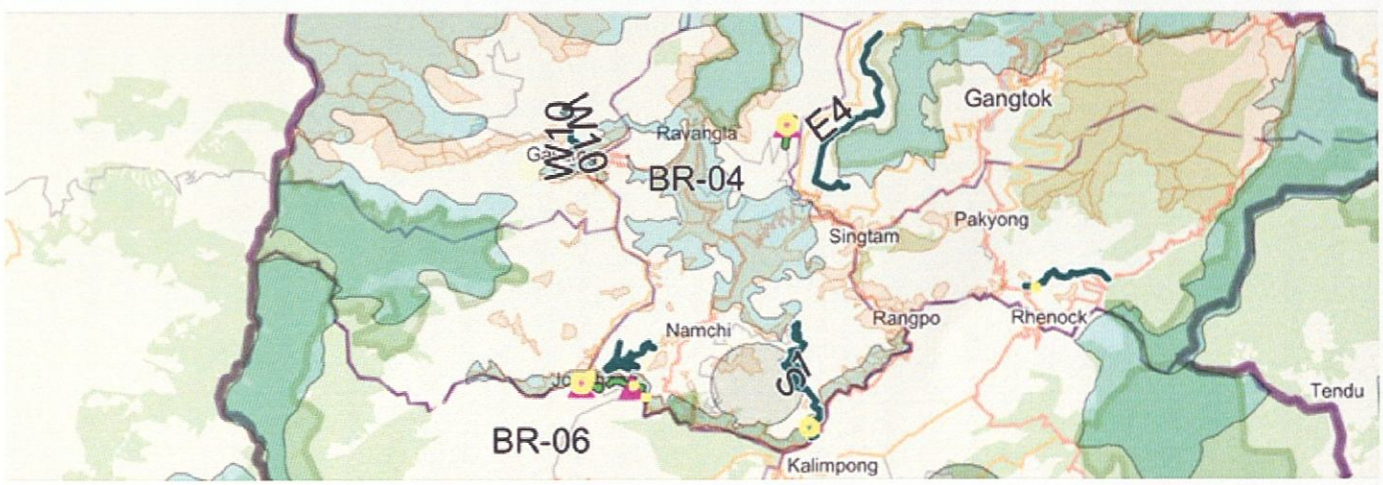

STUDY SITE

SAMPLE COLLECTION

Water samples were collected from six sampling sites in Sikkim. Sample collection was carried out from six different streams with replicates from each site. Samples were collected considering all the variables that will highlight spatial distribution of *Tor putitora* in the waters enhancing the magnitude of species detection probability (Pant *et. al.* in communication).

Sampling at selected sampling sites

The sampling sites were chosen based on plossible sites of road construction near the streams. These sites were selected considering small streams with a depth less than 1m, the presence of algal blooms, sandy substratum, crevices behind the boulders, as these are likely to be preferable breeding grounds and the chances of encountering mahseer presence is higher (Khwairakpam *et al*. 2023). From each of these sites, water samples were collected in sterile gallons from each sampling location. To avoid contamination sterile gloves, collection gallons, surgical and collection tubes were kept aside prior the collection process. After reaching the filtration facility, samples were kept at −4 °C until further processing.

Samples were collected along with variables (Temperature and depth of the sampling location)

SAMPLE FILTRATION

The filtration was carried out using passive filtration method (Deiner *et al.*, 2015). Aseptic conditions were maintained throughout the process of filtration, preservation, and processing of eDNA. Water samples were filtered using MF-Millipore™ Mixed Cellulose Esters (MCE) Membrane Filters Merck, with the help of a vacuum pump (VCP-8101).

For each sampling site, 15- 20 litres of water samples (along with the replicates) were filtered. The membrane filters from each filtered water sample were transferred to the vials containing buffer and were then stored at 4°C. Further processing of the preserved samples (membrane filters) was conducted in the laboratory in aseptic conditions.

SAMPLE PROCESSING AND EXTRACTION

DNA Extraction was performed using DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany), for DNA extraction and the quality of the extracted DNA was checked on a 0.8% agarose, and its concentration was measured using a Qubit® 2.0 Fluorometer.

DNA extraction and PCR of extracted samples in the lab

PCR AND SEQUENCING

The DNA samples were amplified, targeting the COI region of 313 bp, which was utilized for the identification of aquatic macroinvertebrates (Deiner et al., 2013; Hajibabaei et al., 2011).

Library Preparation

The resulting amplicons were processed and utilized for preparing the library. Paired-end sequencing libraries were prepared using the TruSeq Nano DNA Library Prep Kit. A high-fidelity amplification step was performed with HiFi PCR Master Mix to optimize yields from limited starting material. The amplified libraries were analyzed using an Agilent Bioanalyzer 2100 using a High Sensitivity (HS) DNA chip, following the manufacturer's guidelines.

Cluster Generation and Sequencing

The library was loaded onto the Illumina platform after determining its Qubit concentration and mean peak size from the Bioanalyzer profile for cluster generation and sequencing.

POST PROCESSING

Trimmomatic v0.39 (Bolger *et al.*, 2014) and PEAR v0.9.6 (Zhang *et al.*, 2014) were employed to remove adapter sequences and merge paired-end reads, respectively. To minimize false genus identification and mitigate overestimation, similar sequences were clustered using CD-HIT v4.8.1 (Weizhong & Godzik, 2006; Fu *et al.*, 2012).

To refine taxonomic identification, a local BLAST search was performed with BLAST+ v2.5.0 (Camacho *et al.*, 2013) against a curated Tor putitora database. Default blastn parameters were adjusted to retain critical metrics such as sequence length, e-value, and bit score, ensuring accurate resolution of ties among multiple matches.

The reference database included the genus distribution of *Tor putitora* across sampled locations, following the checklist by Baruah *et al.* (2018), and Goswami *et al.* (2012). This approach enhanced match precision and minimized cross-identification errors. BLAST results were systematically analyzed to determine the most accurate match for each query sequence.

RESULTS

Environmental DNA (eDNA) metabarcoding analysis confirmed the presence of *Tor putitora* in selected stream locations within Sikkim. Out of the six sampling sites—

i) BR_04,

ii) BR_06 (Ramam Bridge),

iii) BR_08 (Matizar-Kitam Bridge)

iv) BR08_S (a stream 130 meters from BR_08)

v) T (Teesta Main Stream)

vi) Malli, positive detections were recorded at BR_06 and BR_08, with high certainty (>99%).

These findings confirms the occurrence of *Tor putitora* in specific stream habitats, aligning with its known distribution in the region.

CONCLUSION & DISCUSSION

The identification of *Tor putitora* in selected sites corresponds with the published study regarding the distribution of mahseer in the Eastern Himalayan region. Studies conducted by Goswami *et al.* (2012) and Baruah *et al.* (2018) have highlighted the ecological importance of *Tor putitora* within Himalayan River systems, noting its affinity for clear, swiftly flowing streams characterized by rocky substrates. Additionally, these investigations have recorded a decrease in its populations attributed to habitat fragmentation, overfishing, and environmental alterations.

The detection of *Tor putitora* at sites BR_06 and BR_08 indicates that these locations may offer appropriate habitats for the species, thereby aiding its survival in the face of ongoing ecological pressures, inculding hydropower and linear infrastructure developments. This study adds to the expanding evidence concerning mahseer distribution in Sikkim and highlights the critical need for conservation initiatives aimed at safeguarding its essential habitats, by incorporating appropriate mitigation measures. Continued research and long-term monitoring are imperative to evaluate population dynamics and ensure the sustainable management of *Tor putitora* in the region.

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