Final Report

On

A Multi-Disciplinary Assessment of Biodiversity and Socio-Economic Status of the Karnali River of Nepal

GRANT NO: G-KAT-012

Prepared by

Center for Molecular Dynamics Nepal

USAID
FROM THE AMERICAN PEOPLE

CENTER FOR MOLECULAR DYNAMICS - NEPAL

Published by: Centre for Molecular Dynamics Nepal (CMDN)

Available from: Center for Molecular Dynamics-Nepal , Swaraj Sadan
Prasuti Griha Marg, Thapathali-11, Kathmandu 44600.

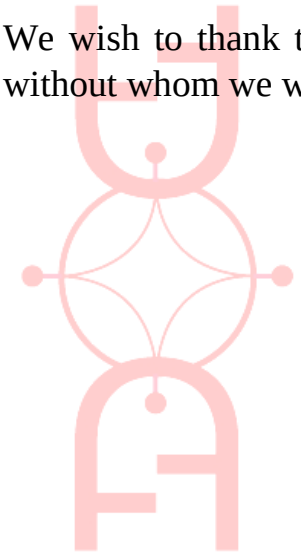
Inside Photographs: CMDN

Acknowledgement

We thank all the field researchers and team members including fishermen and resident local citizens who participated in the field work, Himalayan Biodiversity and Climate Change Center for providing us with the field gear. Special thanks to the Department of National Parks and Wildlife Conservation (DNPWC), and Department of Forest for the permission and field permits.

The contribution of the laboratory researcher's for the development of the protocols and data analysis is highly appreciated. We would like to thank all the authors of this document, our financial team and administrative teams for their ongoing support. A special note of thanks to the PAANI team, Allen Turner, Nilu Basnyat, Deepak Rijal, Suresh Wagle, John Go, and Binita Shrestha for your immense support and guidance in the course of this project.

We wish to thank the residents of the Karnali river Basin for their hospitality and insight without whom we would not have been able to complete this work.



नेपाल सरकार
वन तथा वातावरण मन्त्रालय
राष्ट्रिय निकुञ्ज तथा वन्यजन्तु संरक्षण विभाग
(.....शाखा)

फोन नं. : ४२२०८५०
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फ्याक्स नं. ४२२७६७५

संकेत नं. :-
पत्र संख्या :-
चलानी नं. :- २६०९

पो. ब. नं. - ८६०
बबरमहल, काठमाडौं
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मिति: २०७५/१/२०

श्री बर्दिया राष्ट्रिय निकुन्ज कार्यालय
ठाकुरद्वार, बर्दिया।

विषय: अध्ययन अनुमति सम्बन्धमा।

प्रस्तुत बिषयमा नेपाल कृषि अनुसन्धान परिषदको सहकार्यमा Center for Molecular Dynamics- Nepal (CMDN) ले कर्णाली नदिमा " Biodiversity and Socio-economic Assessment of the Karnali River" कार्य गर्न अनुमति माग गरेको हुनाले सो सम्बन्धमा यस बिभागबाट मिति २०७५/१/१९ गते को निर्णय अनुसार तपसिलको शर्तमा रही उक्त अध्ययन अनुसन्धान गर्न तपाईलाई मिति २०७५/१/२१ देखि २०७५/२/१५ सम्मको लागि अनुमति प्रदान गरेको व्यहोरा अनुरोध छ।

तपसिल

१. अनुसन्धानकर्ताले राष्ट्रिय निकुन्ज तथा वन्यजन्तु संरक्षण ऐन २०२९ र नियमावली २०३० तथा यस मातहतका सबै नियमावालिहरुको पूर्ण पालना गर्नु पर्ने छ।

२. अनुसन्धानकर्ताले विभाग र सम्बन्धित संरक्षित क्षेत्र कार्यालय संग समन्वय गरि कार्य गर्नु पर्ने छ।

३. अनुसन्धानकर्ताले आफ्नो अनुसन्धानको प्रस्ताव सम्बन्धित संरक्षित क्षेत्र कार्यालयमा समेत पेश गर्नु पर्नेछ।

४. अनुसन्धानकर्ताले अनुसन्धान समाप्त भएपछि एक प्रति कागजी प्रतिवेदन र एक प्रति विधुतीय प्रतिवेदन विभाग र सम्बन्धित संरक्षित क्षेत्र कार्यालयमा बुझाउनुपर्ने छ।

५. अनुसन्धानकर्ताले नतिजाहरु प्रकाशित गर्दा अनुसन्धानमा संलग्न कर्मचारीको योगदानको आधारमा सह लेखकको रुपमा समावेश गराउनु पर्नेछ।

६. अध्ययनको क्रममा संकलित माछा तथा पानीको नमुना संरक्षित क्षेत्र भित्रको हकमा सम्बन्धित राष्ट्रिय निकुन्ज कार्यालयको प्रत्यक्ष रोहवरमा उक्त कार्य गर्नु पर्ने छ

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९. संकलित नमुना नेपाल भित्रकै प्रयोगशालामा परिक्षण गर्ने र नमुनाको कुनै पनि Genetic अंश विदेश लैजान नपाउने।

१०. संकलन हुने नमुनाको हकमा नियमानुसार लाग्ने दस्तुर समेत तिर्नु पर्ने।

२०७५/१/२०

भुपेन्द्र प्रसाद यादव
सहायक इकोलोजिस्ट

बोधार्थ

श्री नेपाल कृषि अनुसन्धान परिषद: आबश्यक जानकारीको लागि अनुरोध।

श्री श्री Center for molecular Dynamics- Nepal, Thapathali, Kathmandu : आबश्यक जानकारीको लागि अनुरोध

अनुसन्धानकर्ता : श्री विश्व पराक्रम श्रेष्ठ, श्री आदर्श मान शेरचन र दिबेश बिक्रम कर्माचार्य: सम्बन्धित संरक्षित क्षेत्र कार्यालय संग समन्वय गरी अध्ययन अनुसन्धान गर्नु हुन।

नेपाल सरकार
वन तथा वातावरण मन्त्रालय
वन बिभाग
(योजना तथा अनुगमन महाशाखा)

पत्र संख्या : २०७४।०७५
चलानी नं.: १८०

मितिः २०७४।१२।३०

विषय : अनुसन्धान अनुमति सम्बन्धमा ।

श्री Center for Molecular Dynamics Nepal,
थापाथली, काठमाण्डौ ।

प्रस्तुत विषयमा त्यस संस्था मार्फत "Biodiversity Assessment and Socio-Economic Survey of Karnali River Basin" को विषयमा फिल्ड अनुसन्धानका लागि अनुमति उपलब्ध गराईदिनु हुन भनि यस विभागमा दिनु भएको निवेदन साथ प्रपोजल प्राप्त भयो । सो सम्बन्धमा कारवाही हुँदा उक्त प्रपोजलमा उल्लेखित Methodology अनुसार तपसिलको शर्तहरुको अधिनमा रही सम्बन्धित जिल्ला वन कार्यालयसँग समन्वय गरि अनुसन्धान गर्नु हुन निर्देशानुसार अनुरोध छ ।

शर्तहरु

१. अनुसन्धानकर्ताले वन ऐन २०४९ तथा वन नियमावली २०५१, राष्ट्रिय निकुञ्ज तथा वन्यजन्तु संरक्षण ऐन, २०२९ र नियमावली २०३० तथा यस मातहतका नियमावलीहरुको पूर्ण पालना गर्नुपर्नेछ ।
२. माछाको नमुना संकलन गर्दा Cast Nets विधि मात्र प्रयोग गरी गर्नुपर्नेछ ।
३. माछाका नमुनाहरु संकलन गर्दा जिल्ला वन कार्यालयका प्रतिनिधिहरुको रोहवरमा गर्नु पर्नेछ साथै संकलन गरिएका नमुनाहरुको संख्या लगाएत अन्य विवरणहरु जिल्ला वन कार्यालय साथै वन विभागलाई पनि उपलब्ध गराउनु पर्नेछ ।
४. संकलित नमुनाहरु देश वाहिर लैजान पाइने छैन ।
अनुसन्धानको क्रममा प्राप्त भएको जैविक विविधता संरक्षणसँग सम्बन्धित संवेदनशील सूचनाहरु गोप्य राख्नु पर्नेछ । अनाधिकृतरुपमा त्यस्ता सूचनाहरु कसैलाई पनि उपलब्ध गराउन पाइने छैन ।
५. अध्ययन अनुसन्धान कार्य समाप्त भए पश्चात एक प्रति प्रतिवेदन (कागजी तथा विद्युतिय) यस विभागमा पेश गर्नु पर्नेछ ।

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सुजन महर्जन
सहायक वन अधिकृत

बोधार्थः
श्री जिल्ला वन कार्यालय वाजुरा, सल्यान,वर्दिया, बझाङ्ग, डोटी, अछाम, कैलाली, दैलेख, कालिकोट, जुम्ला, हुम्ला, मुगु, डोल्पा, जाजरकोट, रुकुम, बैतडी, सुर्खेतः जानकारी तथा आवश्यक सहयोगका लागि अनुरोध छ ।

Contents

1 Executive summary

A Multi-Disciplinary Assessment of Biodiversity and Socio- Economic Status of the Karnali River of Nepal was one year full scale project (2018-2019) awarded by DAI Global, LLC PAANI USAID. The project was successfully executed by the Center for Molecular Dynamics Nepal (hereon referred to as CMDN) under the USAID Grant: G-KAT-012.

CMDN conducted in depth genetic and environment assessment of the aquatic biodiversity of the Karnali river basin. CMDN carried detail assessment of the field sites with available primary geospatial mapping and genetic analysis of the samples using DNA barcoding and eDNA meta-barcoding technology. eDNA analysis has the capability to detect and identify species that cannot be identified using traditional fishing catchment. Environmental DNA as a relatively new bio-assessment method has the potential to improve species detection capacity and efficiency compared to traditional capture or observation-based sampling approaches. The major advances of eDNA technology can be described as an application-ready tool that can assist fisheries specialists to achieve research, managements and conservation goals.

CMDN has developed a comprehensive genetic database enlisting details of all the identified fish species. The study recorded a total of 50 species of fish via genetics and eDNA metabarcoding in one year (dry-wet season) of sampling.

The study recognized the need to update existing knowledge of aquatic biodiversity and record the information in the form of visual databases. Aquatic database of the Karnali river basin provides a bird's eye view of the overall fish species identified per sites and interlink with water quality, socio-economic surveys and bio-geographic conditions of the sampling areas. CMDN has also developed an interactive geographic information system (GIS) where field collected information has been integrated to analyze, manage, and present spatial and geographic data of the sampled sites.

CMDN organized a workshop with stakeholders and partners to gain collective support from stakeholders, and address key policy makers in the hydropower and biodiversity sector.

As a part of the agreement CMDN assisted National Agricultural Research Centre (NARC) and PAANI with the setup items required for the molecular biology lab setup at NARC. CMDN hosted a molecular techniques training to NARC researchers on improving their fish genetics capacity. One of the main agenda of the agreement was to develop genetic database for the museum stored fish specimen at FRD, which faced technical challenges due to the nature of the dessicant.

Furthermore, a more comprehensive study of aquatic biodiversity needs to be established to follow up and update knowledge on fisheries, hydrology and to develop measures to strike a balance between development projects and conservation efforts. The outcome of the proposed study would serve as an important tool to answer key questions in regards to conservation and EIAs of hydropower development.

2 Introduction

Biodiversity conservation is an emerging as well as a critical topic in the current development scenario. According to the United Nations Conference on the Environmental and Development (UNCED) held at Rio de Janerio (Brazil) in 1992, 'Biodiversity means the variability among living organisms from all sources including, inter alia, terrestrial, marine and other aquatic ecosystems and the ecological complexes of which they are part; this includes diversity within species, between species and of ecosystems.' People have started taking steps towards biodiversity conservation due to their realization that biodiversity erosion might threaten the very existence of life on earth. (Chandrakar, 2012).

Aquatic biodiversity forms a part of the global biodiversity and includes plants and animals from crayfish to catfish, from mussels to mayflies, from tadpoles to trout (Helfrich et al., 2012). Knowledge on distribution and ecology of native species of Nepali rivers is very limited although these information is essential for ecological management and conservation of the species (NFBP, 2016).

Artificial obstruction plays an important role in providing threats to freshwater biodiversity and recently, hydropower dam construction has taken place at an unprecedented rate which has led to disruption of dynamic processes and ecological integrity of natural systems (McCartney et al., 2000). Hydrological alterations and changes due to dam constructions have contributed more towards collapsing of riverine fisheries than pollution and destructive fishing (UNEP, 2002). Habitat destruction in both feeding and breeding grounds has been caused due to development projects eventually leading to biodiversity loss (ADB, 2018). Also subsistence fishing is supported by aquatic resources and help in generating income through ecotourism, sport fishing and small- scale aquaculture as a wide range of goods and services as well as income-generating opportunities for local people including ethnic groups are provided by the rich diversity of plants and animals in wetlands (ICIMOD, 2004). Thus, disruption of aquatic biodiversity also significantly affects the socio-economic aspects of the country. It is important to use cutting edge sciences in order to elucidate the existing scenario of our current baselines, be it the effect of anthropological activities on the environment or to gauge a true measurement of existing biodiversity. Without metrics, conservation practices may not be as effective and impactful because baseline information provide reference points for the cause and effects of development. Therefore, we have sought to use innovative non-invasive technologies like environmental DNA to detect and characterize fish biodiversity through water samples complimented by traditional capture fisheries.

Nepal's river systems have a tremendous hydropower potential which is estimated to be over 83,000MW- provides ample scope for energy security and economic prosperity. This river provides habitats for a variety of aquatic species distributed from river stretches right from the low to higher elevations. The Karnali, Initial assessment revealed that at least 121 indigenous fish species are inventoried having both ecological and

commercial values in Karnali. There is a lack of comprehensive information on the extent and distribution of fish diversity, our effort has been insufficient to effectively monitor change as well as mitigate the impacts of stressors on the persistence of aquatic diversity *in situ*. (Smith et. al, 1996)

CMDN implemented over two seasonal samples, the first eDNA sampling survey in Nepal's largest river system Karnali citing a crucial lack of aquatic biodiversity baseline information to mitigate against possible development projects that might challenge its future discourse. Regarded as one of the most pristine river basins of the world, we used both capture fisheries and eDNA bypassing a molecular and genetic pipeline to create Nepal's most comprehensive aquatic genetic database consisting of biodiversity, socio-economic, local capacitance, policy implication and GIS mapping onto a holistic platform that would allow us to make well informed and evidence based conservation decisions. This report outlines these variables with available metrics to measure pre implementation, implementation and post-implementation impacts to measure long term sustainability and viability.

3 Field sampling

Field sampling was conducted for both Dry (pre-monsoon, Phase I) and Wet season (post-monsoon, Phase II)

3.1 Pre-monsoon sampling (Phase I)

Field sampling was deployed and conducted starting May 14 2018 to first week of June 2018. Of the 15 sites noted, only P1 (Thuli Bheri confluence) was inaccessible through multiple approach points (by road and/or air) due to unavailable tickets (only cargo planes flying and domestic passenger airlines halted temporarily)

3.2 Post-monsoon sampling (Phase II)

Wet season field sampling was completed between September 1st week to October 1st week. The team deployed of 14 individuals including eDNA, fisherman, fish morphology, water quality assessment and social survey.

The following field information were collected during the same period during field sampling:

a) Fish Capture
b) eDNA sampling
c) Water Quality information
d) Correspondence analysis surveys
e) GIS/Bio-geographical condition information

Table 1: Site information and GPS location

1	Thuli Bheri Confluence	Sampling site	82.87112778	28.97496667	Paani Proposed
2	Confluence at Tila Watershed	Sampling site	81.58386667	29.13945833	Paani Proposed
3	Rupsagad	Sampling site	81.56566944	29.1262	Paani Proposed
4	Humla Karnali	Sampling site	81.75612222	29.31306667	Paani Proposed
5	Nagmagad Sinja Cofluence	Sampling site	81.91363889	29.20164722	Paani Proposed
6	Pulu Area, Mugu Karnali	Sampling site	82.40144167	29.57531944	Paani Proposed
7	Jayaprithivi	Sampling site	81.19639722	29.54865833	Paani Proposed
8	Downstream of Jayaprithivi	Sampling site	80.91080833	29.44332778	Paani Proposed
9	Barapata	Sampling site	80.80480833	29.30016111	Paani Proposed
10	Seti Nadi/Karnali Confluence	Sampling site	80.98164444	28.94207778	Paani Proposed
11	Sanfe bagar	Sampling site	81.21167778	29.22750556	Paani Proposed
12	Maila Area	Sampling site	81.7885	29.62284722	Paani Proposed
13	Thadhawa Gaun	Sampling site	82.16134444	28.613525	Paani Proposed
14	Jhula	Sampling site	82.42519167	28.641025	Paani Proposed
15	Jajarkot Khalanga	Sampling site	82.27293333	28.69166667	Paani Proposed

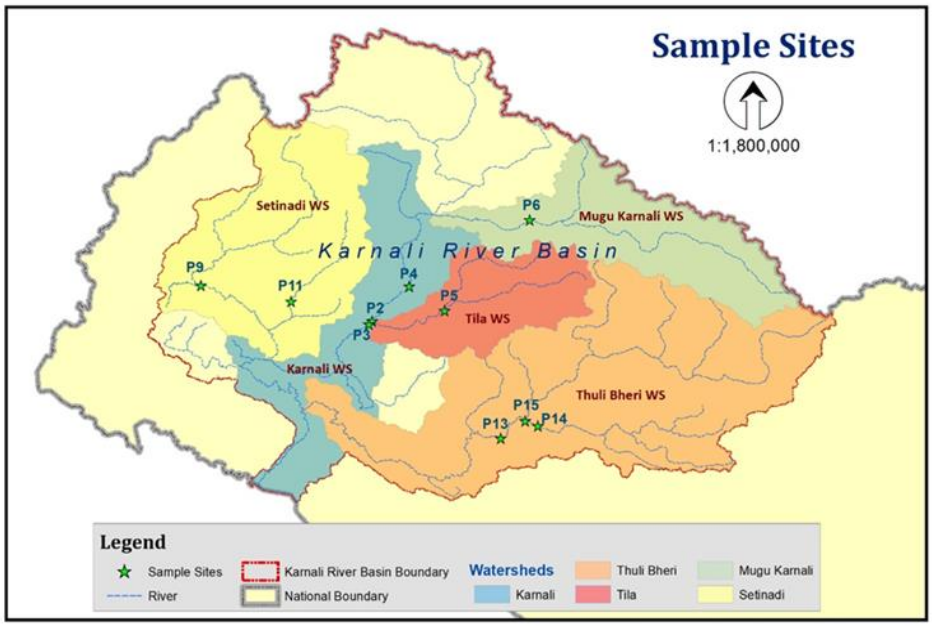

Fig 1: Sampling site location in Karnali River Basin

4 Method and Materials

Field Sampling

4.1 Fish sampling

Cast nets of mesh size 10 mm/20 mm were used for capturing fish. Fish sample were directly weighed, morphologically identified by our fish expert, photographed and recorded. Taxonomic identification was assigned to genus level in majority cases; few samples were identified up to species level.

Fig 2: Cast nets were primarily used for fish collection.

4.2 eDNA sampling:

2L water samples were collected from fifteen different (5 sub-sites) sites distributed along Karnali river basin. In each site, four subset of sample were collected at a 100 meter distance inclusive of one pool and one riffle, upstream and downstream and sediment sites. Prior to sampling, collection bottles were decontaminated by exposing to 10% bleach solution and rinsed by 70% ethanol for 5 min each to remove any residue of DNA on the collection bottle. Water samples were immediately filtered through 47 mm, 0.45µmpore size nylon filter (GE Healthcare, Whatman Millipore) and stored in Longmire solution (Yamamoto, S. et. al, 2017). Deionized water was filtered simultaneously in each site to check for cross contamination. Filter funnels and forceps were bleached after every filtration to minimize contamination.

Fig 3: eDNA sample processing unit

4.3 Socio-Economic Correspondence analysis:

The research team conducted brief social surveys among the locals from different study sites regarding their knowledge on the local names, migratory patterns, spawning, growth, feeding habits, use values, season and additional comments on the fish species sampled from the respective sites.

5 Laboratory Processing

5.1 Tissue Dissection and DNA Extraction

Tissue from the caudal peduncle region was dissected from the collected tissue samples for DNA extraction using Gene All Tissue Mini kit following the instructor's manual. The samples selected were based on morphology representatives of different fish species or their subsets.

5.2 COI Fragment Amplification

The partial COI segment of mt DNA was targeted for DNA barcoding using cocktail mix of fish specific primers (Table 2) which amplified ~650 bp region of the gene (Ivanova et. al, 2007).

Table 2: Primers used for COI amplification

Target gene	Primers ID	Sequences(5' - 3')	Band size(bp)	Reference
COI	VF2_t1	TGTAAAACGACGGCCAGTCAACCAACCACAAAGACATTGGCAC	~ 650bp	(Ivanova et al., 2007)
	FishF2_t1	TGTAAAACGACGGCCAGTCGACTAATCATAAAGATATCGGCAC		
	FishR2_t1	CAGGAAACAGCTATGACACTTCAGGGTGACCGAAGAATCAGAA		
	FR1d_t1	CAGGAAACAGCTATGACACCTCAGGGTGTCCGAARAAYCARAA		
	M13F(-21)	TGTAAAACGACGGCCAGT		
	M13R (-27)	CAGGAAACAGCTATGAC		

A total of 25µL PCR final reaction was prepared containing 12.5 µL of 2X Qiagen multiplex master mixes, 2.5 µL of 5X Q-solution, 0.25 µL 10pMol/ µLFish COI Cocktail primer sets and 2 µL of extracted DNA. The thermocycling (MJ Research Tetrad PTC-225 Thermal Cycler, USA) condition was 95 ºC for 15 min followed by touch down PCR of 5 cycles of 94 ºC for 60 sec, 48 ºC for 50 sec and 72 º C for 50 sec followed by 35 cycles of 94 ºC for 60 sec, 50 ºC for 50 sec and 72 º C for 50 sec with the final extension at 72 º C for 5 min. Amplified ~ 650 bp target PCR product was visualized on Gel-Doc, (Major ScientificTM) under 2% agarose gel electrophoresis.

In total, 172 (81 from phase I and 91 from phase II) positively amplified tissue samples were processed further for forward and reverse sequencing.

5.3 Sequencing

The amplified PCR products were sequenced using Big Dye Terminator Kit Version 4.1 for further analysis. Positive amplified PCR products were purified using enzymatic clean up (ExoSAP-IT) removing unconsumed dNTPs and primers. Sequencing reactions were performed in a MJ Research PTC-225 Peltier Thermal Cycler using a ABI PRISM® BigDyeTM Terminator Cycle Sequencing Kits with AmpliTaq®

DNA polymerase (FS enzyme) (Applied Biosystems), following the protocols supplied by the manufacturer. Single-pass sequencing was performed on each template using species specific both forward and reverse primers. The fluorescent-labeled fragments were purified from the unincorporated terminators with BigDye® XTerminator™ purification protocol. The samples were re-suspended in distilled water and subjected to electrophoresis in an ABI 3730xl sequencer (Applied Biosystems).

5.4 Sequence analysis and Species Identification

The sequencing of the PCR products were performed at both forward and reverse directions. The two sequence reads of each sample were processed for trimming followed by assembling via AliView software (Larsson 2014). The assembled sequences were subjected to BLAST against reference NCBI GenBank database for taxonomic assignment. For species identification, a threshold of 97% nucleotide identity was used for conservative confirmation with reference database.

ENVIRONMENTAL DNA (eDNA)

5.5 eDNA extraction from water samples

Environmental DNA (eDNA) was extracted from the filter membranes using Gene All Tissue Mini kit following the instruction manual following minor modifications. Negative controls were run in parallel during extraction procedure to monitor potential contamination.

5.6 Amplicon library preparation and Mi-Seq sequencing

Two step PCR protocol was used to prepare Illumina MiSeq dual indexed amplicon libraries (Miya et al., 2015). Template-specific primers with 5' Illumina tails were used for first round PCR amplification followed by purification using Agencourt AMPure magnetic bead. A second round amplification was performed using Illumina adapters with Nextera indexes (Nextera® XT Index Kit (96 indexes, 384 samples and Nextera index v2index kit). The six random hexamers (N) were used to enhance cluster separation on the flowcells of MiSeq sequencing platforms (Miya et al., 2015).

Two step PCR protocol of using primers with identical tails in the first step and indexed primers in the second, is a specifically developed by Illumina to reduce bias caused by variable index sequences in mixed environmental samples (Berry et.al, 2011, Donnell et.al, 2016).

The final products were pooled, purified with AMPure beads and quantified using a HS Qubit assay (Thermo Fisher Scientific). Amplicons were normalized (4nM) and sequenced (loading concentration 10pM) on an Illumina MiSeq instrument using a MiSeq reagent kit v2 2 × 150 bp (Illumina, Singapore cycles).

5.7 Data pre-processing and analysis

The Illumina MiSeq generated the paired-end reads as de-multiplexed fastq files (forward and reverse reads) for each sample from a single NGS library run. Before performing any analysis, a quality control checks about overall quality of the raw sequence reads was evaluated by program FastQC, available from Babraham Bioinformatics (https://www.bioinformatics.babraham.ac.uk/projects/fastqc). After the quality control procedures, the sequence data were processed in metabarcoding framework using the software packages in OBITools, an open-source python-based package specifically designed for analyzing NGS data in a DNA metabarcoding context (Boyer et al., 2016). A data analysis pipeline was developed in python programming language that incorporates OBITools scripts to perform series of sorting, filtering and taxonomic annotations in automated mode in Linux server for processing raw sequence data of all the samples. Firstly, the paired-end reads were assembled into single reads while unaligned reads were removed. The assembled reads were then processed to trim out primer sequence regions from its either ends and tagged with sample code as its attribute. The reads were then dereplicated into unique sequences while retaining information about read counts in each sample as sequence attributes. This step was mainly performed to reduce both file size and computations time as it is convenient to process unique sequences instead of multiple reads of the same DNA sequence. The next step involved denoising of the sequence dataset that may contain PCR and/or sequencing errors or chimeras. In this filtering step, rare sequences and sequence variants were removed that likely correspond to artifacts and were not necessarily biologically meaningful.

5.8 Taxonomic assignment

Finally, after all the filtering steps were completed, the refined sequence dataset was assigned to corresponding taxonomic species by matching against a reference NCBI GenBank database using BLAST software (Madden, 2013). Performing BLAST remotely against online GenBank database can be time consuming, thus a BLAST database of GenBank was setup locally in Linux server, which allows standalone BLAST to be operated through command-line. This version is much faster and efficient for processing large datasets using a single command. This step generated the complete list of fish species associated to each sample. For taxonomic assignment, sequence identity threshold of 97% match was used in order assign species. A final Operational Taxonomic Unit (OTU) table was generated with all identified fish species along with their corresponding read counts in each sample.

6 Results

6.1 Capture Fish and eDNA

Out of 172 positively amplified samples (phase I, N=81, phase II, N=91), **26** species of fish were identified **t**hrough genetic sequencing of Cytochrome-Oxidase-I (COI) gene by comparing sequence reads against the NCBI database.

Table 3: The following total list of fish were identified through fishing efforts. Of the total positively amplified fish for sequencing (N=172) from phase I (N=81), phase II (N=91), the following 26 listings were identified.

PAANI Final Fish Capture Phase I and II															
SN	*sscinames*	Count	P02	P03	P04	P05	P06	P07	P08	P09	P10	P11	P13	P14	P15
1	*Acanthocobitis botia*	1								1					
2	*Barilius barna*	1									1				
3	*Barilius bendelisis*	12							1	2	1	2	2	2	2
4	*Barilius vagra*	7		1					1	2			2		1
5	*Botia lohachata*	2										1		1	
6	*Channa gachua*	2											2		
7	*Crossocheilus latius*	13		1					1	3	1	2	1	2	2
8	*Garra cf. annandalei CTOL3904*	8								5		2		1	
9	*Garra sp. 1 SK-2014*	13	2	1					2	1	1		2	2	2
10	*Glyptothorax pectinopterus*	2										1		1	
11	*Glyptothorax gracilis*	1									1				
12	*Glyptothorax trilineatus*	1								1					
13	*Labeo bata*	8							1		1	2	2	1	1
14	*Labeo boggut*	9							1	1	2	3		1	1
15	*Mastacembelus armatus*	1								1					
16	*Neolissochilus hexagonolepis*	8							2	2		1		3	
17	*Opsarius cf. shacra CTOL02808*	8									4		2	2	
18	*Pseudecheneis sulcata*	2		2											
19	*Puntius chelynoides*	5	1	1	1					1		1			
20	*Aspidoparia morar*	2									2				
21	*Schistura savona*	1								1					
22	*Schizothorax sp.*	39	1	5	3	2	5	4	4	2	2	4	2	3	2
23	*Schizothorax plagiostomus*	6	1	1	1				1	1	1				
24	*Schizothorax progastus*	9	2	1	1		1	1			1			1	1
25	*Schizothorax richardsonii*	1										1			
26	*Tor putitora*	10								3	4	2		1	
	Grand Total	172	7	13	6	2	6	5	14	27	22	22	15	21	12

Table 4: The following total list of 27 fish species were identified through phase I of eDNA sampling efforts enlisted below

PAANI Final Operational Taxonomic Unit Above 200 Phase I										
SN	*sscinames*	Total	P2	P3	P4	P6	P7	P9	P12	P15
1	*Barilius sp. CBM ZF 11313*	3641065	465288	1007560	904168	597941	182223	91191	392588	106
2	*Schizothorax sp*	2318133	274958	679028	84332	267939	721332	1988	288501	55
3	*Barilius bendelisis*	1539297	137872	175434	21373	67671	55639	269890	811397	21
4	*Garra sp. CBM ZF 11369*	633324	584156	34123	112	3197	1492	3643	6601	0
5	*Pethia conchonius*	409856	32800	66515	87132	60946	31583	1	130872	7
6	*Schistura corica*	258935	78977	70700	43491	3043	1340	6821	54551	12
7	*Channa punctata*	236231	53	34	67913	29247	0	1	138933	50
8	*Tor putitora*	114479	5553	29634	32011	21034	18133	0	8114	0
9	*Puntius jerdoni*	107159	39	65101	42016	3	0	0	0	0
10	*Triplophysa dorsalis*	103975	74	93480	32	6738	2	0	3649	0
11	*Labeo boggut*	68629	24	68599	6	0	0	0	0	0
12	*Hypophthalmichthys nobilis*	33573	5	0	0	0	0	0	33567	1
13	*Pseudecheneis sulcata*	31461	31366	69	24	0	1	0	0	1
14	*Acanthocobitis botia*	30508	19574	22	14	0	0	0	10898	0
15	*Schistura rupecula*	8952	0	0	0	0	0	4476	4476	0
16	*Triplophysa sp. 3 YW-2016*	1270	0	11	1	0	0	0	1258	0
17	*Glyptothorax cavia*	421	418	3	0	0	0	0	0	0
18	*Cyprinus carpio*	227	2	9	27	33	1	0	0	155
19	*Carassius auratus*	127	0	20	0	4	0	0	0	103
20	*Botia lohachata*	33	0	33	0	0	0	0	0	0
21	*Pterophyllum scalare*	12	0	3	0	1	0	0	0	8
22	*Cirrhinus cirrhosus*	8	0	0	1	0	0	0	0	7
23	*Channa striata*	6	0	0	1	0	0	0	0	5
24	*Carassius auratus x Cyprinus car*	5	0	2	0	0	0	0	0	3
25	*Puntius sophore*	2	1	0	0	0	1	0	0	0
26	*Carassius gibelio*	2	0	1	0	0	0	0	0	1
27	*Clarias gariepinus*	2	0	0	0	0	0	0	0	2
		7652326	1631162	2290388	1282657	1057801	1011758	378011		549

Table 5: The following total list of 21 fish species were identified through phase II of eDNA sampling efforts.

PAANI Final Operational Taxonomic Unit Above 200 Phase II																
SN	sscinames	count	P2	P3	P4	P6	P7	P8	P9	P10	P11	P12	P13	P14	P15	P16
1	*Barilius sp. CBM ZF 11313*	4634304	389898	632540	667358	242886	228886	92842	106494	12	724166	346108	404594	451492	13638	333390
2	*Schizothorax sp.*	1399610	141248	157572	191428	132	254124	108394	65536	10	76654	80	152348	111432	18520	122132
3	*Barilius bendelisis*	725138	62376	38010	47860	35270	26916	6294	132564	2	40504	44452	59390	205878	10	25612
4	*Tor putitora*	260002	21926	5114	99900	96	5844	2438	4714	2	45804	28198	20	664	2	45280
5	*Schistura corica*	222082	36688	9938	37048	70	0	0	8898	0	13432	7904	2906	80410	1422	23366
6	*Pethia conchonius*	116382	0	30	2	0	0	0	156	115988	180	0	0	0	0	26
7	*Schistura rupecula*	99826	12704	6	2	0	0	0	2128	0	14	24	84934	14	0	0
8	*Neolissochilus hexagonolepis*	73088	18226	10	12	0	0	0	54816	2	18	0	0	4	0	0
9	*Labeo boggut*	64674	2	0	0	0	0	0	42198	2	17490	0	0	1138	2704	1140
10	*Puntius sophore*	64488	42832	16	19906	74	0	0	2	0	2	0	0	2	0	1654
11	*Garra sp. CBM ZF 11369*	42996	12	10380	5186	16	0	0	2822	0	20008	6	8	12	4	4542
12	*Triplophysa tibetana*	32840	32796	22	22	0	0	0	0	0	0	0	0	0	0	0
13	*Puntius jerdoni*	24768	0	0	0	2	5202	0	19002	2	6	0	0	554	0	0
14	*Cyprinus carpio*	15324	2	0	0	15310	2	0	0	0	6	0	0	0	0	4
15	*Glyptothorax trilineatus*	7638	0	0	2	0	0	0	7634	0	2	0	0	0	0	0
16	*Puntigrus tetrazona*	5496	0	0	0	0	0	0	5492	0	4	0	0	0	0	0
17	*Channa punctata*	5298	8	4616	2	0	0	0	10	0	0	0	0	0	0	662
18	*Botia lohachata*	1414	0	0	0	0	0	0	1414	0	0	0	0	0	0	0
19	*Pseudecheneis sulcata*	898	0	0	0	0	0	0	896	0	2	0	0	0	0	0
20	*Glyptothorax cavia*	812	0	0	0	0	0	0	0	0	0	0	0	0	808	4
21	Schistura sp. CBM:ZF:15598	20	6	0	0	0	0	0	4	0	0	0	10	0	0	0
		7797098	758724	858254	1068728	293856	520974	209968	454780	116020	938292	426772	704210	851600	37108	557812

Table 6: Total Fish identified through eDNA and fish catchment methods

SN	Species	Designation	Count
1	*Barilius sp. CBM ZF 11313*	eDNA & Fish	
2	*Tor putitora*	eDNA & Fish	
3	*Labeo boggut*	eDNA & Fish	
4	*Pseudecheneis sulcata*	eDNA & Fish	
5	*Acanthocobitis botia*	eDNA & Fish	
6	Neolissochilus hexagonolepis	eDNA & Fish	
7	Glyptothorax trilineatus	eDNA & Fish	
8	Botia lohachata	eDNA & Fish	
9	*Schizothorax sp.*	eDNA & Fish	
10	*Barilius bendelisis*	eDNA & Fish	
11	*Garra sp. CBM ZF 11369*	eDNA & Fish	11
12	*Pethia conchonius*	eDNA only	
13	*Schistura corica*	eDNA only	
14	*Channa punctata*	eDNA only	
15	*Puntius jerdoni*	eDNA only	
16	*Triplophysa dorsalis*	eDNA only	
17	*Hypophthalmichthys nobilis*	eDNA only	
18	*Schistura rupecula*	eDNA only	
19	*Triplophysa sp. 3 YW-2016*	eDNA only	
20	*Glyptothorax cavia*	eDNA only	
21	*Cyprinus carpio*	eDNA only	
22	Puntius sophore	eDNA only	
23	Triplophysa tibetana	eDNA only	
24	Puntigrus tetrazona	eDNA only	
25	*Carassius auratus*	eDNA only	
26	*Pterophyllum scalare*	eDNA only	
27	*Cirrhinus cirrhosus*	eDNA only	
28	*Channa striata*	eDNA only	
29	*Carassius auratus x Cyprinus carpio x*	eDNA only	
30	*Puntius sophore*	eDNA only	
31	*Carassius gibelio*	eDNA only	
32	*Clarias gariepinus*	eDNA only	
33	Schistura sp. CBM:ZF:15598	eDNA only	22
34	*Barilius barna*	Fish only	
35	*Barilius vagra*	Fish only	
36	*Channa gachua*	Fish only	
37	*Crossocheilus latius*	Fish only	
38	*Garra cf. annandalei CTOL3904*	Fish only	
39	*Garra sp. 1 SK-2014*	Fish only	
40	*Glyptothorax pectinopterus*	Fish only	
41	*Glyptothorax gracilis*	Fish only	
42	*Labeo bata*	Fish only	
43	*Mastacembelus armatus*	Fish only	
44	*Opsarius cf. shacra CTOL02808*	Fish only	
45	*Puntius chelynoides*	Fish only	
46	*Aspidoparia morar*	Fish only	
47	*Schistura savona*	Fish only	
48	*Schizothorax plagiostomus*	Fish only	
49	*Schizothorax progastus*	Fish only	
50	*Schizothorax richardsonii*	Fish only	17

6.2 eDNA from the pristine Himalayan lakes

We extended our high tech eDNA technology from the western river to some of the Nepal's most pristine high altitudes fresh water lakes in the Himalayas. Lakes located in the High Mountains are glacial in origin and are poorly inhabited with aquatic life (ADB, 2018). Among the high altitude lakes, fish fauna is reported only in Rara Lake. Other lakes such as, Shey Phoksundo in Dolpa are considered oligotrophic with low primary productivity. We processed few extra samples ~2L from some of the fresh water lakes as listed below.

Table 7. Water samples from Himalayan lakes

Khaptad	Provided by PAANI team
Shey Phoksundo	Provided by PAANI team
Parshuram	Provided by PAANI team
Rara	Collected by CMDN

Among the samples processed, we were only able to pool the eDNA collected from Rara and Phoksundo. The remaining samples didn't meet minimal DNA concentration requirement (4 nM) hence were ommitteed from the sequencing run. eDNA concentration measured in those samples were very low to detect (<1nm) and to be processed further in downstream analysis(Table 8).

Table 8. List of species identified by eDNA metabarcoding in Rara and Phoksundo

SN	sscinames (Rara)	Rara: P16
1	*Barilius sp. CBM ZF 11313*	333390
2	*Schizothorax sp.*	122132
3	*Barilius bendelisis*	25612
4	*Tor putitora*	45280
5	*Schistura corica*	23366
6	*Labeo boggut*	1140
7	*Puntius sophore*	1654
8	*Garra sp. CBM ZF 11369*	4542
9	*Channa punctata*	662

	sscinames (Phoksundo)	DPH1
1	*Barilius sp. CBM ZF 11313*	229116
2	*Pethia conchonius*	88416
3	*Garra sp. CBM ZF 11369*	83099
4	*Schistura corica*	52135
5	*Barilius bendelisis*	16579
6	*Tor putitora*	1452
7	*Schizothorax nepalensis*	232

6.3 Socio-economic survey analysis

Socio and economic survey was conducted by CMDN field team in all the listed fifteen sites. A set of questions and key points regarding their knowledge on the local names, migratory patterns, spawning, growth, feeding habits, use values and season was discussed with the local people. The aim was to obtain qualitative as well as quantitative information from locals on the basis of fish consumption, seasonal, religious culture value and habitat degradation.

Use value of fish differed between people based on seasons. For example: they specifically preferred pancreas and bile of Mahasser (*Tor putitora)* during summer as good source of protein, providing nutrition subsistence and supplemental income to the hilly population (Sarma et al. 2015). In an unpublished study by Smith et al (1996), sampling conducted during the low water season in the Karnali river identified Mahseer (*Tor putitora*), Mrigal carp (*Cirrhinus mrigala*), and Sucker catfish (*Bagarius bagarius*) as the species that held the highest value for local artisanal fisherman. Mahseer (*Tor putitora*) which has been listed as endangered, are now even more vulnerable and are declining in a steady state (Smith et al. 1996). Majority of the people in the area are involved in fishing at the subsistence level (Malla, 2009). Apart from the normal cast nets, gill nets, hook line, they also used poison despite being illegal. The use of poison degrades water quality and kills large number of fishes and other aquatic faunal species resulting in the degradation of habitat (Malla, 2009). Anthropogenic activities like fishing, washing, transporting forest products and other river dependent activities done for the livelihood are creating gradual disturbances in an aquatic ecosystem. Similarly, the trend of chemical fertilizers around the river area is increasingly being used thus posing threat to fish ecosystem (Malla, 2009).

Conservation of aquatic biodiversity is an issue that directly concerns the livelihood and quality of life of local people Aquatic species are threatened with local extinction from the effects of habitat degradation, segregation of breeding groups by downstream barrages and incidental catches during fish operations. Strategies to conserve aquatic biodiversity need to be addressed as part of an overall approach that links environmental priorities with economic and social development. (Smith et al. 1996).Therefore to ensure native fish conservation policies need to be put forth in order to restrict or, at least, monitor excessive fishing of such endangered species. (Gurung, T.(2013)

6.4 Voucher specimen Museum

We created an in-house museum, or specimen library, of all representative identified fish species based on Co1 gene sequencing. Out of 26 identified fish, we were able to physically create voucher specimen for 23

individual species. For this, we created a separate lab where all voucher specimens were stored in preservative medium with taxonomic information.

Fig 4: Voucher specimens stored in formalin

7 Capacity Building at NARC and Molecular Training in CMDN

CMDN assisted NARC and PAANI with the setup items required for the molecular biology lab setup at NARC.

CMDN hosted four days of full course basic molecular lab training for NARC as a part of the capacity building. Three researchers from NARC (Prem Timalsina, Suraj Kumar Singh and Prakash Kunwar) were fully trained from 21st March- 25th March 2019 on the basic molecular laboratory techniques (Tissue dissection, DNA extraction, PCR amplification, Gel electrophoresis and basic bioinformatics analysis) as well as machine operation.

Table 9. Participants attending Training in CMDN

NARC MOLECULAR TRAINING (21MARCH-25 MARCH 2019)

DATE	ATTENDIES	EMAIL ID	CONTACT
21 March	Prem Timalsina	p.timalsina01@gmail.com	9842086648
21 March	Suraj Kumar Singh	suraj 9842529211 @gmail com	9842529211
21 March	Prakash kunwar	prakash.kunwar66@gmail.com	9841580235
22 March	Prakash kunwar	"	"
22 March	Suraj Kumar Singh	suraj 9842529211 @ gmail com	9842529211
22 March	Prem Timalsina	p.timalsina01@gmail.com	9842086648
24 March	Prem Timalsing	" "	" "
24 March	prakash kunwar	prakash.kunwar66@gmail.com	
25 March	prakash kunwar		9841580235
25 March	prem Timalsina		9842086645

Fig 5: Participants from NARC during discussion of molecular techniques

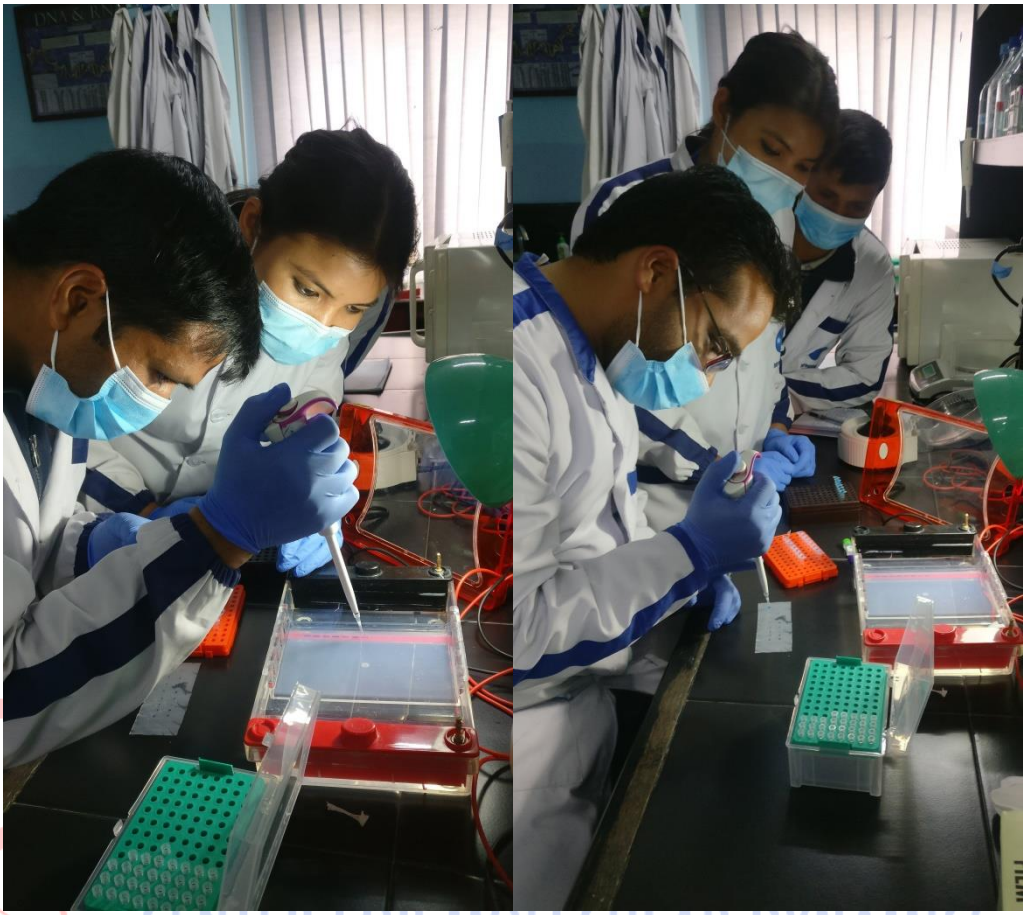

Fig 6. Hands on training of gel electrophoresis during molecular training

Fig 7. Data interpretation of Gel electrophoresis

8 Genetic Database of NARC fish specimen

Among our outlined project goals signed between CMDN and USAID funded PAANI project, one of the listed objective was to develop genetic profile of the fish specimens stored in formalin at Fisheries Research Department, Godavari.

A team of five researchers (Lab team/ field team/ Fish morphological expert) visited FRD to dissect tissue (caudal peduncle) from the museum stored samples.

Fig 8. Museum Speciem stored in Formalin at FDR

Fig 9. FDR staff helping CMDN fish team for sorting museum specimens

Fig 10. CMDN fish team dissecting the selected fish specimen

A total of N=50 tissue samples was collected from the formalin stored voucher specimen to extract DNA and create a genetic reference database for museum stored samples.

We processed a total of 39 samples out of 50 but none of the samples amplified positive. A set of different extraction protocols were implemented to extract high yield DNA from the formalin stored samples. However, isolating high-quality genomic DNA from formalin-fixed PET is difficult because only minimal amounts of intact DNA may be present in the sample (Lin et.al 2002).

Crosslinking is formed between protein and DNA complexes when tissue nucleoproteins is exposed to formalin (Lin et.al 2002).This cross-linking of biomolecules to DNA could directly reduce the PCR amplification efficiency from the genomic DNA of formalin-fixed tissue and therefore significantly decrease the yield of amplifiable genomic DNA (Shapiro et. Al, 2012)

Therefore, we have reported FRD team regarding the issues with formalin stored samples.

Table 10. Summary of different extraction protocols implemented for formalin samples with target gene and amplification results

EXTRACTION METHODS	TARGET GENE	AMPLIFICATION
Gene All- Tissue extraction Kit	CO1, 12S	FAILED
ZYMO Research FFPE KIT	12S	FAILED
Qiagen Modified Protocol	12S	FAILED
Extraction using Alkali treatment	12S	FAILED
PHIX 29 (TempliPhi Protocol)	12S	FAILED
Phenol- Chloroform	12S	FAILED

Full report on FRD museum specimen will be made available separately.

9 GIS/Databases:

GIS data of both field phases I and II (Pre-Post-Monsoon) has been systematically incorporated into a single dynamic multi-data layered map. Field and lab based Geospatial decoding of data is currently available on the weblink:

http://gis.edufinity.net/pages/fish_map?fbclid=IwAR3RTJJrkBssQTj7X5f16gIA6LeR4F7enqTc5urp2wdZ1DvuCisVpRK7BaY

Fig 11: Representation of our GIS multi layered Map

Similarly, CMDN has created a singular database portal enlisting details of all identified species per sites from field to lab of both phases.

We can easily extract the following information through our GIS/database:

1) Fish collected from the field relative to GPS data
2) eDNA collected from field relative to GPS data
3) Water quality data from field relative to GPS data
4) Endangered fish/mammalian species from around sampling sites
5) Socio-economic survey results from sample sites
6) Bio-geographic conditions from sampling areas

10 Stakeholder workshop/meetings

CMDN conducted a local stakeholder workshop on 24th June 2018 at Summit Hotel which consisted of various experts represented from multidisciplinary backgrounds (governmental, hydropower, conservation, academia, donor agencies, and media personnel). Nilu Basnyat, deputy Chief of Party, PAANI Program presented about PAANI's ongoing work at Karnali, current and future scope/objectives of the project. from CMDN presented about the biodiversity assessment through eDNA and genetic technology, Dr. Nita Pradhan from Fisheries Research Department (FRD), NARC presented on NARCs activities in relation to fisheries development and Mr. Ramesh Bhushal (Nepal Environmental Journalists Forum) spoke about the importance of media and outreach programs in promoting projects such as PAANI. The workshop concluded in 3 breakout groups discussing a 6 step process that involved strengthening processes of research data defined but not limited by:

1) The political context surrounding the issue
2) The Stakeholders involved
3) Internal behavioral changes applied
4) Integration strategies among stake holders
5) Internal capacity of the project to effect change
6) Monitoring and overseeing plans

Fig 12: Participants attending Stakeholder Workshop organized by CMDN

Fig 13.Dr Deep Narayan Shah stating main outcome of the group discussion

All three groups presented each of their findings at the end of the 45 minute discussion breakout session. The main resulting outcome of the workshop showed that no single policy advocacy can be conducted by an individual, a multidisciplinary effort to resolve such knowledge to policy efforts are increasingly necessary. More prolonged and progressive dialogue among expert stakeholders was increasingly necessary.

A stakeholder meeting among all grantees under the PAANI program was organized at PAANI office premises where we presented our findings (field and lab) of the dry season (May-June) field work. Potential outcomes, methodologies and future implications of this research were also shared among participating grantee members as part of information exchange.

Other grantees also presented their findings and a joint discussion between all partner organisations on PAANI's goal objectives (through multi-disciplinary research modules) was discussed. It was also agreed upon that regular and timely updates from each grantee would be shared among all participating members through and open and transparent communication network to maximise the learning outcomes of the PAANI project. Addressing this to key policy makers in the hydropower, biodiversity sector was further discussed as was concluded that Nepal was under the developmental narrative where development and conservation needed to go hand in hand.

It was concluded that research and policy need to address each other's needs intricately by assessing how research can aid into policy implementation and how policy drives priority research activity. However, it was also identified that policy needs to go through a strict political process and not a standalone research mechanism for it to be implemented on various levels of governance.

11 Poster Presentation and Data dissemination at 3rd National River Summit:

CMDN presented at the 3rd National River Summit at Rakam Karnali its scientific outcomes and possibilities of environmental DNA for evidence based research, conservation implications for sustainable hydropower development. The research was presented amongst development sector experts as well as conservation experts and the provision of an innovative technology like eDNA that measures or allows an evidence based metric to witness the true impact of development on conservation.

USAID FROM THE AMERICAN PEOPLE

Third National River Summit 2019
Rakkam Karnali
28th-31st March 2019

CENTER FOR MOLECULAR DYNAMICS - NEPAL

Comparison of aquatic biodiversity of two Nepali river basins- with and without hydropower development, using baseline fish diversity data obtained through environmental DNA (eDNA)

Authors

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Abstract

Background
Understanding the diversity, distribution and abundance of fish species is key to effective river systems management. In Nepal, hydropower development offers ample energy and economic opportunities but at the potential cost of depleting its local indigenous fish population.In our study, we used novel environmental DNA (eDNA) technology to compare the fish biodiversity and relative abundance between the species of Karnali river with low hydropower activity and Trishuli with over 12 planned or operational hydropower projects. In doing this, we attempt to uncover the impact of hydropower dams on fish abundance through quantifiable eDNA reads represented by Operational Taxonomic Units (OTU). "OTU" generally refers to clusters of (uncultivated or unknown) organisms, grouped by DNA sequence similarity of a specific taxonomic marker gene.

Methodology

eDNA sampling and extraction
2 litre water samples were collected from 15 sites (P1-P15) at Karnali & 7 (EF1-EF1) sites at Trishuli (5 sub-sites each). Water samples were immediately filtered through 47 mm, 0.45µmpore nylon filter (GE Healthcare, Whatman Millipore) and stored in Longmire solution. Environmental DNA (eDNA) was extracted from the filter membranes using Gene All Tissue Mini kit following the instruction manual.

Amplicon Library and MiSeq sequencing

12S rRNA mitochondrial gene fragment was amplified using MIFISH Universal primers (Miya et al. (2015)). Two step PCR based approach was used to construct paired end libraries for Illumina sequencing (Illumina 16S Metagenomic Sequencing Library preparation guidelines, Illumina, Inc., San Diego, California, USA)

Data Processing/Analysis/Taxonomic assignment

Fastq files generated by Illumina MiSeq, upon undergoing quality tests were processed through a data analysis pipeline using OBITools (using Python coding). Paired reads were assembled to single reads that were further trimmed. Quality check included removal of error sequences and chimeras. The refined sequences were aligned against NCBI GenBank database (through local servers) and sequence matches above 97% were accepted to assign a species represented in their individual Operational Taxonomic Units (OTU).

Fig 1: (L-R) Top: Environmental DNA sample collection, eDNA sample field filtration processing, Polymerase Chain Reaction (PCR) of target 12S mitochondrial gene, Next Generation Sequencer (NGS) results **Fig 2 : (L-R) Bottom:** eDNA OTU quantity differences represented atfree flowing Karnali and hydropower influenced Trishuli.

Results

A checklist of 33 fish species were identified from Karnali and 25 spedes from Trishuli through eDNA. The genera with higher eDNA abundances (particularly *Barilius, Schizothorax, Garra*) occupied close to 60%, 15% and 10% of their respective sampling pools (Karnali wet, Karnali dry, Trishuli) showing similar relative abundance consistencies. *Barilius sp.* OTU numbers in Karnali were about 8.604 times higher than in Trishuli, *Schizothorax sp.* OTU were about 12.4309 times higher than Trishuli and *Barilius bama* OTU numbers were about 21 (21.0086) times higher. The overall cumulative OTU values at Karnali were 8 times higher than at Trishuli. Traces of mayfly (*Epeorus sp.*) eDNA was also discovered from Karnali samples. Hydropower activity in Trishuli is much more rampant (with multiple actively operating projects) than Karnali, which for now is still a free flowing river.

eDNA Operational Taxonomic Unit (OTU) counts of highly abundant fish species at each site in Karnali (dry and wet season) and Trishuli (Dry season). A comparative presence/absence and relative abundance illustration between two river systems affected by hydropower development.

sblastnames	OTU Reads	OTU %
bony fishes	15712764	94.686%
mayflies	710986	4.284%

Fig 3: Top : (L-R)
eDNA reads (Operational Taxonomic Units *OTU) for *Barillus sp.* wet and dry season are higher in Karnali river (1.5 million reads) compared to 200,000 reads in Trishuli. **Bottom:** Mayfly population detected through eDNA reads at Karnali, often indicative of non-polluted rivers.

Discussion

In our study, of the most relatively abundant genera from both pools, *Schizothorax* & *Barilius* genera, were close to 8 to 12 times lower at Trishuli compared to Karnali. Higher eDNA concentration is linked with greater fish biomass, thus indicating possibility of greater fish populations represented by greater OTU quantity. Hydropower dams are known to change river morphology; water depth, discharge and obstruct pathways of migratory fish which causes rivers to augment and create low quality habitat for aquatic biota. The Trishuli river, currently in mode to operate over 12 hydropower projects indicates the trend of augmenting low quality habitat for fish to breed, migrate and survive, hence represented by low OTU numbers. Moreover, Karnali eDNA OTU is represented by about 4% mayfly populations (*Epeorus sp.*) which is highly indicative of biodiversity rich, non-polluted river systems further adding to the case that Karnali with lower hydropower disturbance has richer fish biodiversity than Trishuli. 11% of the freshwater fish species in the Amazon rivers are known to be under direct threat of population decline due to dam obstruction. The direct impact to migration pattern, breeding habits, water chemistry, seasonal dynamics or production value brought about hydropower construction needs to be further understood in in lowered eDNA concentrations and thus quantifiable OTU values.

Conclusion

Our study highlights the significance of using a modern rapid monitoring tool like eDNA through the use of Next Generation Sequencing (NGS) to highlight river conservation awareness. Measures are needed to assess and curb the expansion of destructive dams and mitigate their negative impacts. To maintain ecosystem integrity, and sustainable utilization of river resources, we argue that hydropower projects are designed such that these will have minimal impacts on the maintenance of the freshwater biodiversity. Furthermore, the use of baseline monitoring tools like eDNA should be applied more frequently so baseline data can reach saturation point and quantitative as well as qualitative assessment of pre & post cause and effects of hydropower development can be monitored and mitigated with the correct conservation strategy.

Acknowledgements:
We would like to acknowledge and thank the USAID PAANI Project for funding this study. The International Finance Corporation (IFC) for use of EIA based research data. CMDN's field and lab/data analysis teams who have performed all of the field/wet and dry lab work.

Fig 14: Poster presentation represented at 3rd National River Summit, Karnali.

12 Discussion

Baseline information on biodiversity is one of the most essential components required for larger environmental management processes. An appropriate frame of reference or baseline information is necessary against which evaluation is made while setting objectives for any conservation activities or judging their efficacy after implementation (Bull et al., 2014). No proper use of reference baseline data has proven to be a problem for contemporary conservation (Ferraro and Pattanayak, 2006 and McDonald-Madden et al., 2009). When there is lack of knowledge about biodiversity states prior to the rise of harmful anthropogenic activities, the consequences of such pressures cannot be fully understood which also leads to poor implementation of appropriate conservation goals and strategies (Mihoub et al., 2017).

To collect these baselines, our data has shown that whilst traditional fishing methods are effective for real-specimen based assessment of aquatic biodiversity, they are often hindered by the limitations of time-factor, cost factor and logistics. Furthermore, hampered in their inability to detect low abundance species. Environmental DNA meta-barcoding approach is highly reliable and cost effective for the amount of information it can generate within a limited reach of time. eDNA, as a new emerging non-invasive technology is used extensively to genetically monitor not only the aquatic biodiversity but overall biodiversity in an ecosystem. We were able to barcode all our identified species using 12S and COI genes now in part process register as part of NCBI (National Centre for Biotechnology Information) universal database, which is a first for Nepal. The study showed that the Karnali river is inhabited by *Barilius sp*, Schizothorax sp, *Tor putitora* as well as other important species to Nepal. Of these and other listed species, *Tor putitora and Neolissochilus hexagonolepis* are listed under the International Union for Conservation of Nature (IUCN) Red List as "endangered" and "near threatened," respectively. Similarly, *Cyprinus carpio*, *Cirrhinus cirrhosis*, and *Puntius chelynoides* are listed under "vulnerable" per the IUCN red list.

Our findings have underlined that eDNA is effective in collecting extensive information of aquatic inventory, whereas traditional method sometimes being very labour intensive provides complete but limited information regarding aquatic biodiversity in the river systems. Therefore, to detect, lowly abundant, cryptic species, it is clear that just traditional fisheries may be insufficient, hence, complimenting technologies like eDNA helps in creating true baselines of the species available.

Furthermore, we were able to synchronize all our field to lab data onto a singular platform (GIS and Database) that will become for the years to come, a reference portal to track, add and update all fisheries related information for relevant stakeholders (government, research, academia) to access. These baseline

databases therefore can be used to enter information regarding existing as well as new river basins and become the largest repository of fisheries data in Nepal.

13 Conclusion:

A singular year of sampling along Nepal's largest river system was a challenging ask, however, with innovative technologies, we were able to overcome these hindrances faced by many research groups in the past and successfully assess a large biodiversity metric with our DNA technology. With this, we were able to barcode 50 fish species through just two seasons (1 year of sampling) and raise many important questions about the possible trade-offs for future development projects bound to affect these species. Among them, many migratory species that travel upstream to breed as part of its natural life cycle, many that travel downstream to feed. Should there be significant blockage in their pathways through human interference, these species, their communities and their future health and existence should be taken into strict consideration before implementing any related development construction. Moreover, Close linkage working with taxonomists is seen as a major improvement area that we could perhaps work further on as eDNA or genetics along with a strong referential taxonomy will provide long term solutions in the identification or lack thereof of newly found or unaccounted species of Nepal. However, this study has proven the viability of eDNA as a rapid and sensitive biodiversity monitoring tool and effective as an application for future based projects, especially in hydropower development (EIA). Its non-invasive nature means that in a conservation scenario, we see it being implemented by the development sector as a key monitoring tool be it for aquatic or terrestrial flora and fauna. Furthermore, the samples already collected can tomorrow be used to identify downstreaming levels of flora and fauna (example: Macroinvertebrates, amphibians, plants) as the metabarcoding ability of environmental samples allows it to do so.

In conclusion, it is impossible to assume complete baselines over a singular season of sampling, hence, we believe that furthermore research opportunities should be provided in order to maximize the use of innovative technologies, firstly, to strengthen the sciences behind the technology, and more importantly, to get an accurate gauge of the total existent biodiversity by multiple sampling seasons and efforts. CMDNs efforts on collecting and creating accurate baselines will continue as conservation mitigation strategies only become effective if the metric of baselines are accurate, enabling us to understand our conservation trade-off's in this ongoing environment that is our barrage of development projects.

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