CENTER FOR MOLECULAR DYNAMICS - NEPAL

NEPAL FISH BIODIVERSITY PROJECT

Update Report

May 29, 2016

CENTER FOR MOLECULAR DYNAMICS - NEPAL

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Nepal Fish Biodiversity Project
www.fish.org.np

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Acronyms:

NFBP	Nepal Fish Biodiversity Project
CMDN	Center for Molecular Dynamics Nepal
MoFSC	Ministry of Forest and Soil Conservation
DNPWC	Department of National Parks and Wildlife Conservation
USAID	United States Agency for International Development
INPL	Intrepid Nepal Pvt. Ltd.
CPK	Chisa Pani Karnali
GK	Geruwa Karnali
KK	Katasi Karnali
UCK	Upstream from Chisapaani Karnali
UFK	Upstream Far Karnali
UNK	Upstream near Karnali
DNK	Downstream near Karnali
TMK	Tila Mouth Karnali
UT	Upper Tila
BNP	Bardia National Park
DNA	Di-oxyribos Nuclic Acid
eDNA	Environmental DNA
PCR	Polymerase Chain Reaction

1. Overview:

There is little knowledge about native fish species distribution and ecology, which is essential for ecological management and conservation biology of species. This requires the knowledge about species at population level that can sometimes be at low densities, which usually is based on visual detection and counting (Rees et al, 2014). However, unlike their terrestrial counterparts, relatively little is known about native fish and other aquatic organisms in Nepal. Information on the distribution of native fish in Nepali rivers is limited; and there is a paucity of knowledge about the ecology and genetic and life-history diversity of many species, including those important to people.

Nepal Fish Biodiversity Project (NFBP) implemented by CMDN with collaborative efforts and technical support from USDA Forest Service, University of North Carolina at Asheville, and generous support from USAID. NFBP is a research study especially focusing on the creation of native fish biodiversity database of Nepal. This study utilizes genetic markers and environmental DNA (eDNA) metabarcoding technology which shall be used as reference for future genetic research purpose.

The sites chosen are along the Karnali River where there is availability of aquatic life and possibility of learning the environmental impact on them. This report provides the overall update on what has been accomplished from the project initiation on 01 May 16 till the end of the month on 31 May 16.

2. Site Characterization:

Phase I

First phase visit to the Western Nepal specifically Karnali (Bardia National Park) was carried out in first week of May. Main objective of this initial field visit was to scout sample collection sites. During the trip we conducted a survey in the areas of Chisapani, Thakur & Dwara from 02 May 16 till 06 May 16.

Phase II

Sites with permits and probability of collecting diverse fish species were selected for sampling. Sites were prioritized based on the availability of aquatic life and the impact it has on the environment. The areas selected were around Bardia National Park of Far-Western Nepal where sampling took place starting 01 May 16 to 11 May 16.

S. No	Sampling Sites	Code
1	Chisa Pani Karnali	**CPK**
2	Geruwa Karnali	**GK**
3	Katasi Karnali	**KK**
4	Upstream from Chisapaani Karnali	**UCK**
5	Upstream Far from Upper Karnali Hydropower Project	**UFK**
6	Upstream Near from Upper Karnali Hydropower Project	**UNK**
7	Downstream Near from Upper Karnali Hydropower Project	**DNK**
8	Downstream Far from Upper Karnali Hydropower Project	**DFK**
9	Tila Mouth Karnali	**TMK**
10	Upper Tila	**UT**

3. Fish Sampling Training & Workshop

Field Training for sampling procedures, safety measures was provided by CMDN. The standard protocol was developed (by Drs. Santosh Dulal and Dr.David Gillete) for field sampling of fish and eDNA. Selected Five field support staffs (field interns) from Kathmandu University and Tribhuvan University received training required for field sampling, from CMDN Lab team and Dr. David Gillete, Professor from University of North Carolina, Asheville.

The Training covered:

- Field sample collection/handling.
- Sample Storage
- BSL 2 / Aseptic technique.
- Safety training.

During this workshop, the logistics were planned and all related sampling materials were packed for field visits. Field work started from the first week of 01 May 2016.

4. Sample Collection Technique:

The best sample technique was considered to be Electro fishing and net fishing. Institutional Animal Care and Use Committee and Animal Welfare Act were referred to for humane collection of sample.

- Electro-fishing: As is fairly common today, a scientific survey technique utilized for sampling fish population, to determine the abundance, density, and species composition in aquatic environment such as streams, lakes. Electrofishing uses direct current electricity to capture fishes which results in no permanent harm if the electrofishing procedures are properly performed.
- Net fishing: With the help of local fisher men, nets were used to capture fish in the river site.

Figure 1.1
Field team sorting out fish near Chisapani Karnali

Figure 1.2
Electro fishing with David Gillette from University of North Carolina, Asheville

5. Sample List:

- A total of about 321 native fish samples were collected from all nine sites. Fin samples from 99 fishes were placed in formalin. 190 fishes were placed in 90% ethanol. The rest of 32 samples were placed in formalin as specimen.
- Out of 321 fish samples, 42 different species were characterized based on morphological studies, where most of them are already known species, some unknown species need to be characterized.
- 30 water samples were taken from different sites for eDNA analysis.

Total number of fish samples:

S. No	Area	No of Samples
1	CPK	40
2	GK	60
3	KK	37
4	UCK	39
5	UFK	28
6	UNK	34
7	DNK	42
8	TMK	25
9	UT	16
	Total	321

Sample Storage Condition

S. NO.	Sample stored	No of samples
1	Sample in Ethanol	190
2	Fin cut (sample in Formalin)	99
3	Sample in formalin	32
	Total	321

6. Laboratory Optimization

Laboratory optimization was done for the proper workflow of the project. A new lab was set up at CMDN specifically for eDNA fish project.

Figure 2.1
NFBP's new lab was visited by Mr. Nicolai Stohr from US Forest service.

Figure 2.2:
Few of the NFBP's team members

Optimized workflow:

Sample information was digitized and entered in the NFBP database. Hard copy was filed and internal code was generated
Samples were sorted out, barcoded and stored in cabinets.
Extraction will be done in the Bio-Safety II cabinet.
Species identification will be done on each extracted sample.
All field and laboratory information will be archived in a searchable database being developed by NFBP team.
GIS mapping for each individual sample will be prepared and all results placed in www.fish.org.np database.

7. Project Schedule:

Gantt chart was prepared to formulate the work.

		May-16				Jun-16				Jul-16			
S. No.	**Task**	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**
1	**Field Work**												
2	**Digitize Field Data**												
3	**Barcoding**												
4	**Species Categorization/Sorting**												
5	**Morphological Lab setup**												
6	**Lab work (Fish)**												
7	**Lab work (Water)**												
8	**Result Dissemination**												
9	**Further Work Plan**												

8. Project Key Parameters:

Overall update for the first month of project is On-Track as seen in the following chart.

Schedule	On-Track	The project is 50% complete
Quality	On-Track	Preparation is done as per GLP standards.
Lab work	On-Track	Execution in progress.
Risks	On-Track	No major risk. Proper PPE for formalin is being used.
Issues	On-Track	No major issues.

- Field Data has already been digitized.

Figure 3.1
INPL staff working on a pie chart for all species identified according to digitized field data.

- Characterization and Bar-coding is complete for all 289 ethanol samples.

Figure 3.2: Fish samples in Ethanol sorted and bar-coded.

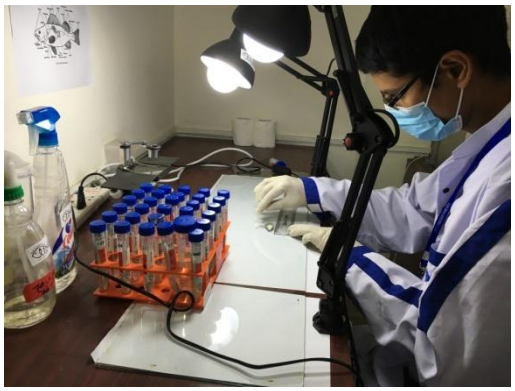

Figure 3.3: Fish morphological characterization

- For the first trial 42 fish samples representing 42 identified species with good morphological condition has been processed for fish dissection.

9. Future project plan:

Formalin sample specimen preserving process will be initiated by June first week. Identified species of fish will be processed for tissue dissection and photography. DNA extraction will be done followed by screening of COI primers. The first priority is given for fish DNA extraction and downstream applications. After the completion of fish DNA works, eDNA metabarcoding will be on the pipeline.