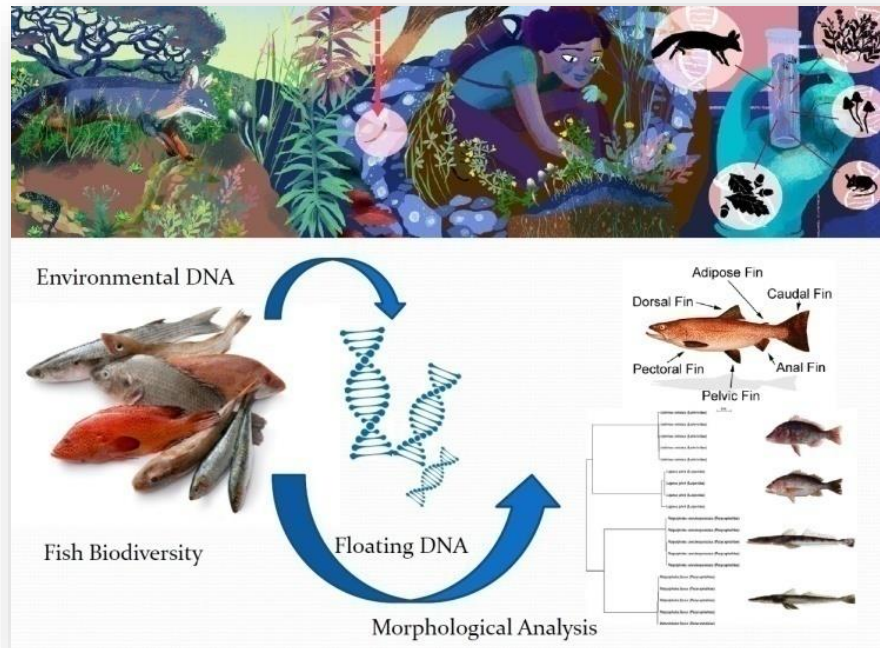


# Ecological and eDNA Survey of Trisuli River for Aquatic Biodiversity Assessment



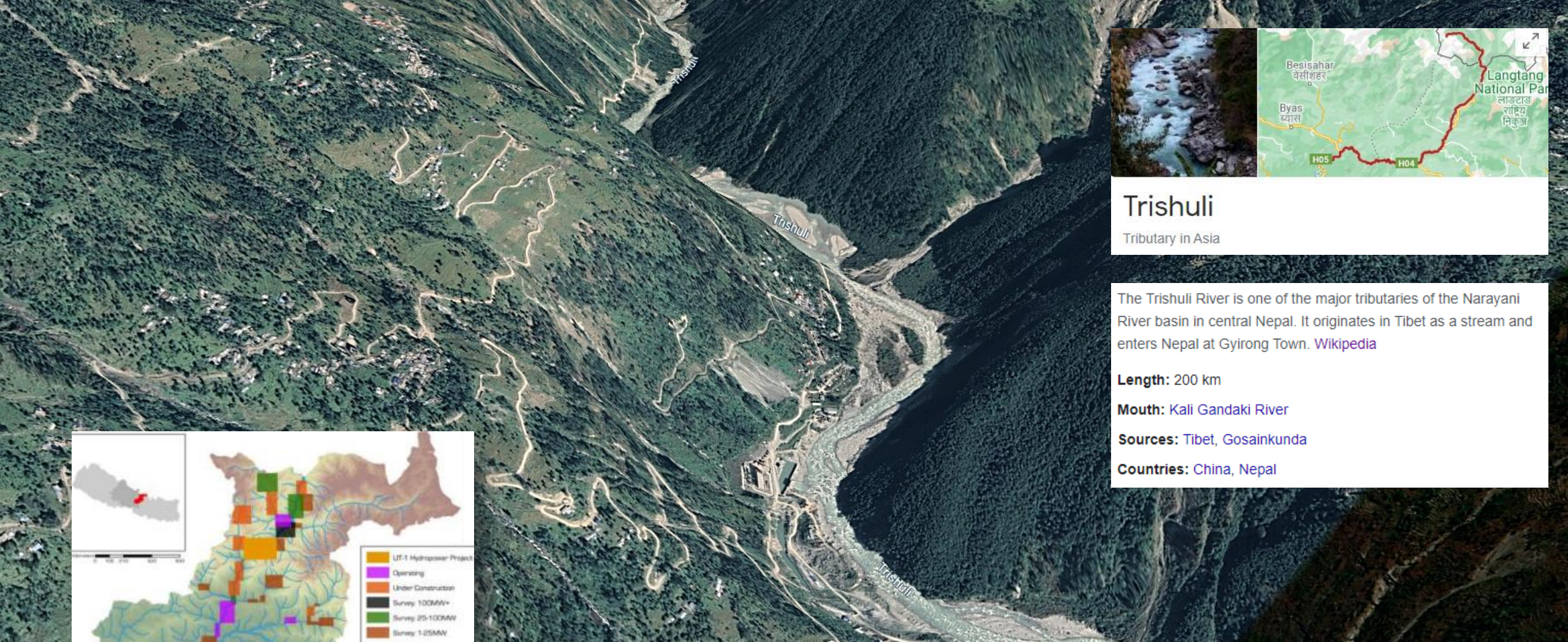
## Presenter:

Dr. Dibesh Karmacharya

Executive Director | Principal Investigator

Center for Molecular Dynamics Nepal





## Trishuli

Tributary in Asia

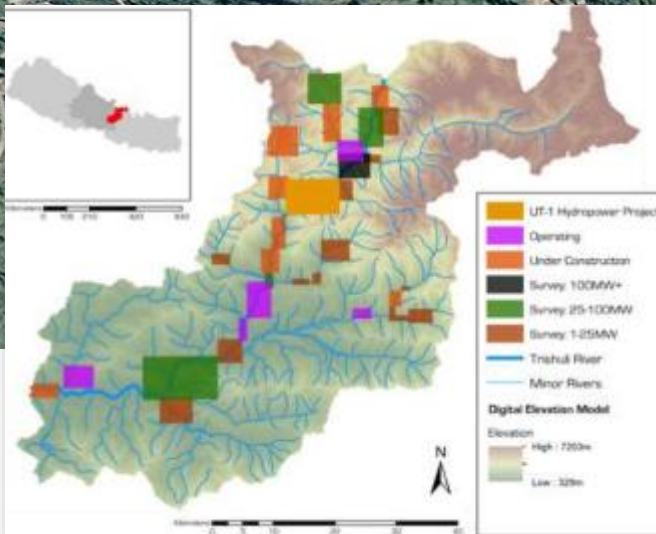
The Trishuli River is one of the major tributaries of the Narayani River basin in central Nepal. It originates in Tibet as a stream and enters Nepal at Gyirong Town. [Wikipedia](#)

**Length:** 200 km

**Mouth:** [Kali Gandaki River](#)

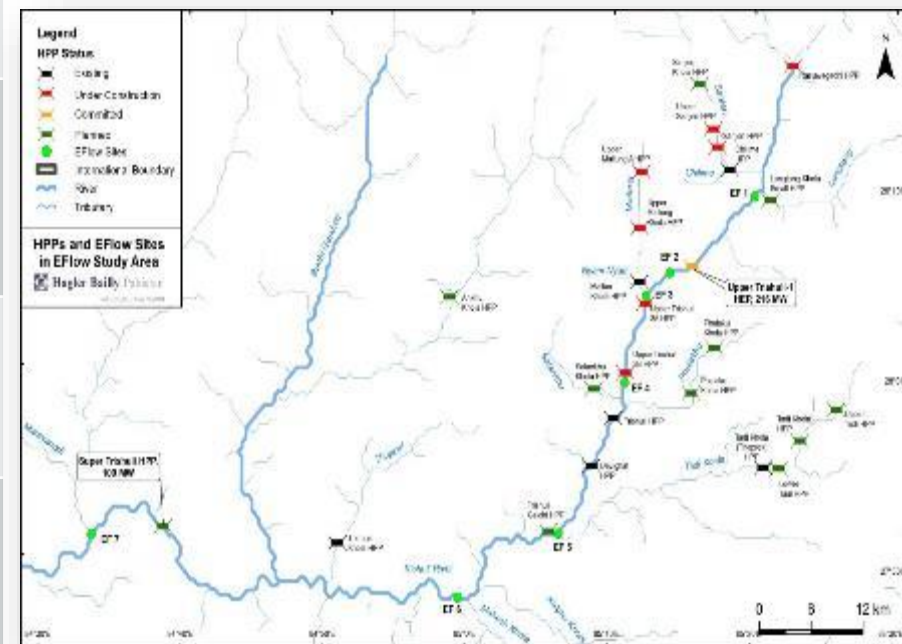
**Sources:** [Tibet](#), [Gosainkunda](#)

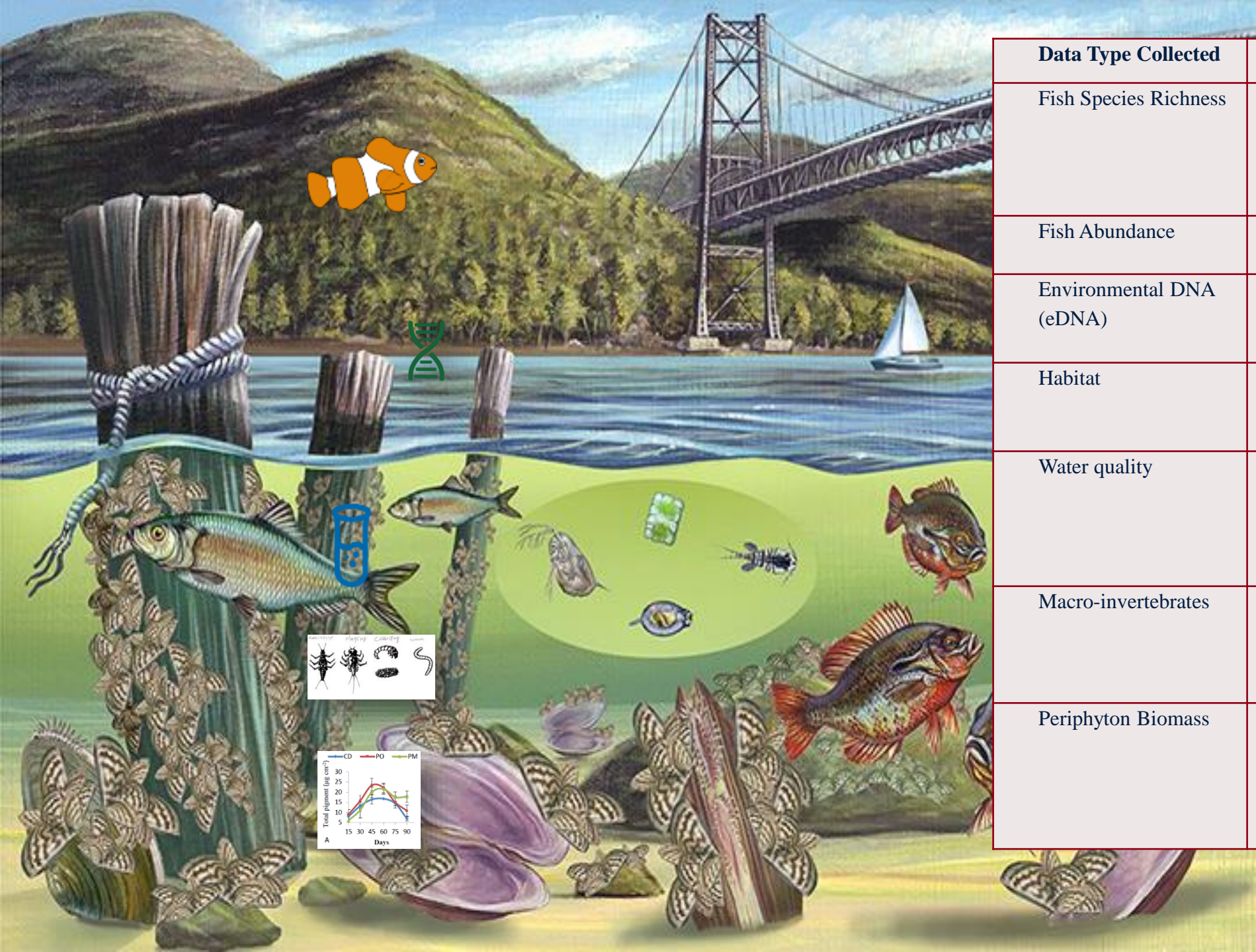
**Countries:** [China](#), [Nepal](#)





SN	Site	Date of Sample collection	General description	Type of River Habitat	Wind	Cloud cover	Type of River Bed	Elevation
1	EF1	2018-10-11	Upstream UT-1HPP(216MW) dam	Pools, Riffles, Rapids	Light	30%	Small cobbles, Large cobbles, Boulders	1430m
2	EF2	2018-10-10	Between UT-1 weir and tailrace	Pools, Riffles, Rapids	Medium	10%	Sand, Small cobbles, Large cobbles, Boulders	1008m
3	EF3	2018-10-09	Downstream of UT-1 tailrace	Pools, Riffles, Rapids, Other/Species habitats	Medium	Sunny	Sand, Large cobbles, Boulders. Maximum depth of river bed(m) 85cm at sampling location	860m
4	Mailing Tributary	2018-10-10	Next to EF-2	Pools, Riffles, Rapids, Other/Species habitats	Medium	100% Haze	Sand, Small cobbles, Large cobbles, Boulders. Maximum depth of river bed(m) 79cm.	993m
5	Langtang Tributary	2018-10-11		Pools, Riffles, Rapids, Other/Species habitats	Light	100%	Sand, Silt, Small cobbles, Large cobbles (Maximum depth of river bed while sampling(m) 54cm.	1430m
6	Bhote Koshi Tributary	2018-10-11		Pools, Riffles, Rapids, Other/Species habitats	Light	100%	Sand, Silt, Small cobbles, Large cobbles	1430m



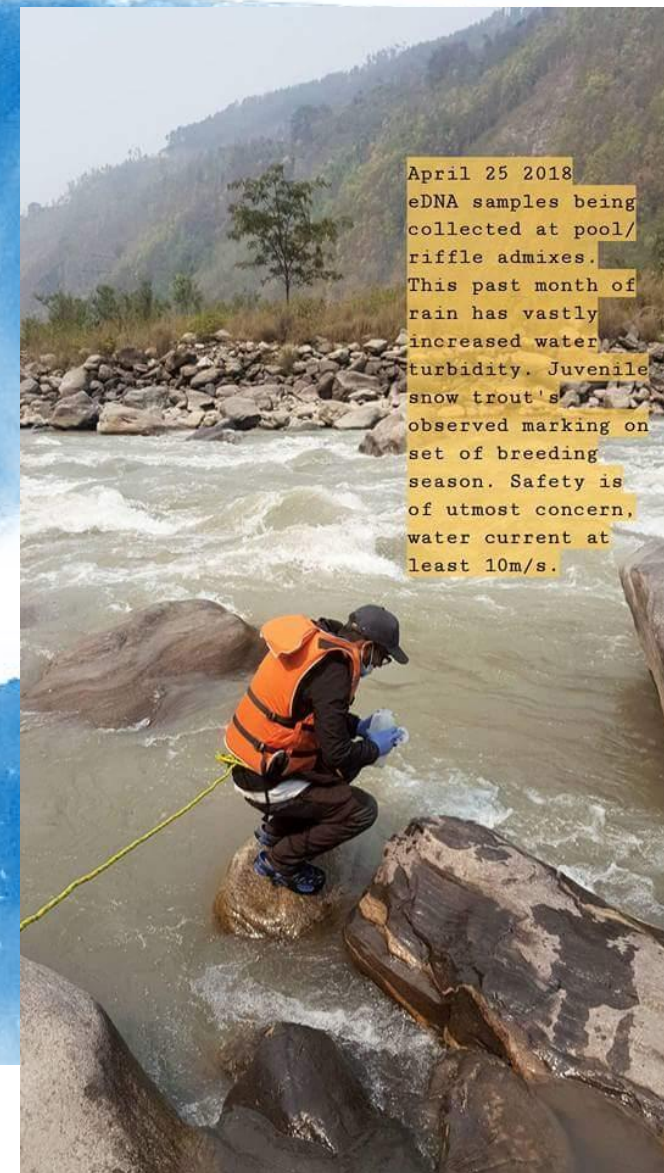
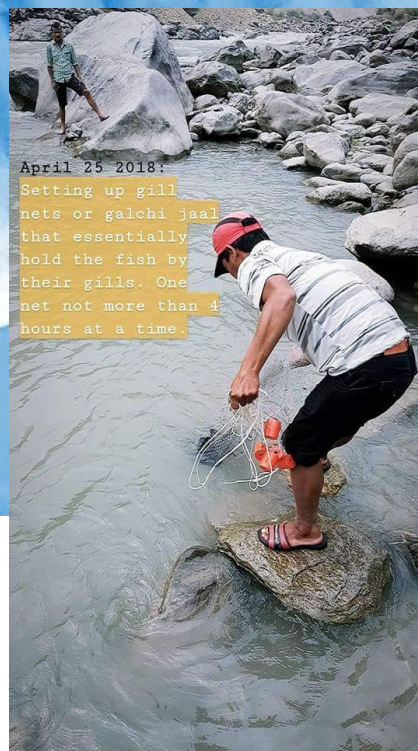


Data Type Collected	Parameters Measured
Fish Species Richness	Genus, species (morphological and genetic identification), voucher specimen collection, fin clips (for DNA extraction)
Fish Abundance	Relative abundance, distribution among sampling sites
Environmental DNA (eDNA)	2 L water sample from each location
Habitat	Relative substrate percent cover, relative vegetation percent cover, dominant habitat types
Water quality	Temperature (°C), pH, total acidity (mg/L as CaCO <sub>3</sub> ), dissolved oxygen (mg/L), CO <sub>2</sub> , total hardness (mg/L as CaCO <sub>3</sub> ), and total alkalinity (mg/L as CaCO <sub>3</sub> )
Macro-invertebrates	Relative abundance, biomass (mg/m <sup>2</sup> )
Periphyton Biomass	Ash-free dry mass (mg/cm <sup>2</sup> ), chlorophyll a (ug/mL)



## Fish Collected

Caught (cast, angling, gill net)	125
Voucher brought to Lab	65
DNA barcoded Species identified	11







Fishing



Water Parameters



eDNA

# Optimized workflow

- Digitization of sample information
- Internal code for samples
- Morphologically characterized samples bar-coded & stored



**Fish samples in preservative**



**Close up analysis of fish for accurate classification**



**Photography and bar-coding sample**

## Nepal FISH Reference Database

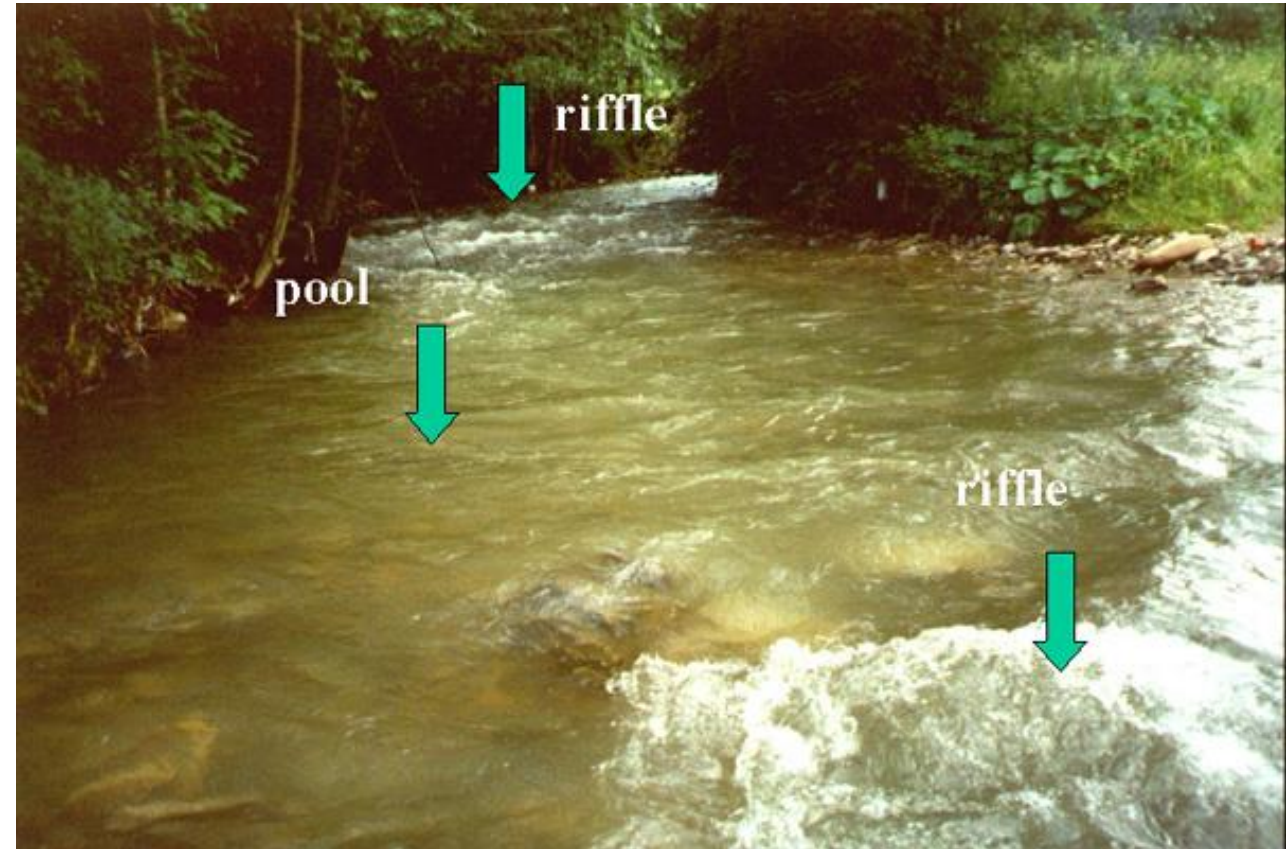






## Field eDNA Sampling Procedure

- Each sample site must be **at least 100 m** in length, and contain at least one riffle and one pool.
- Working from **downstream to upstream**, four aquatic water samples (**D,P,R,U,S**) for each site
- When arriving at a sampling location, put on **latex exam gloves**, remove a 1 L bottle (2 times) from cooler

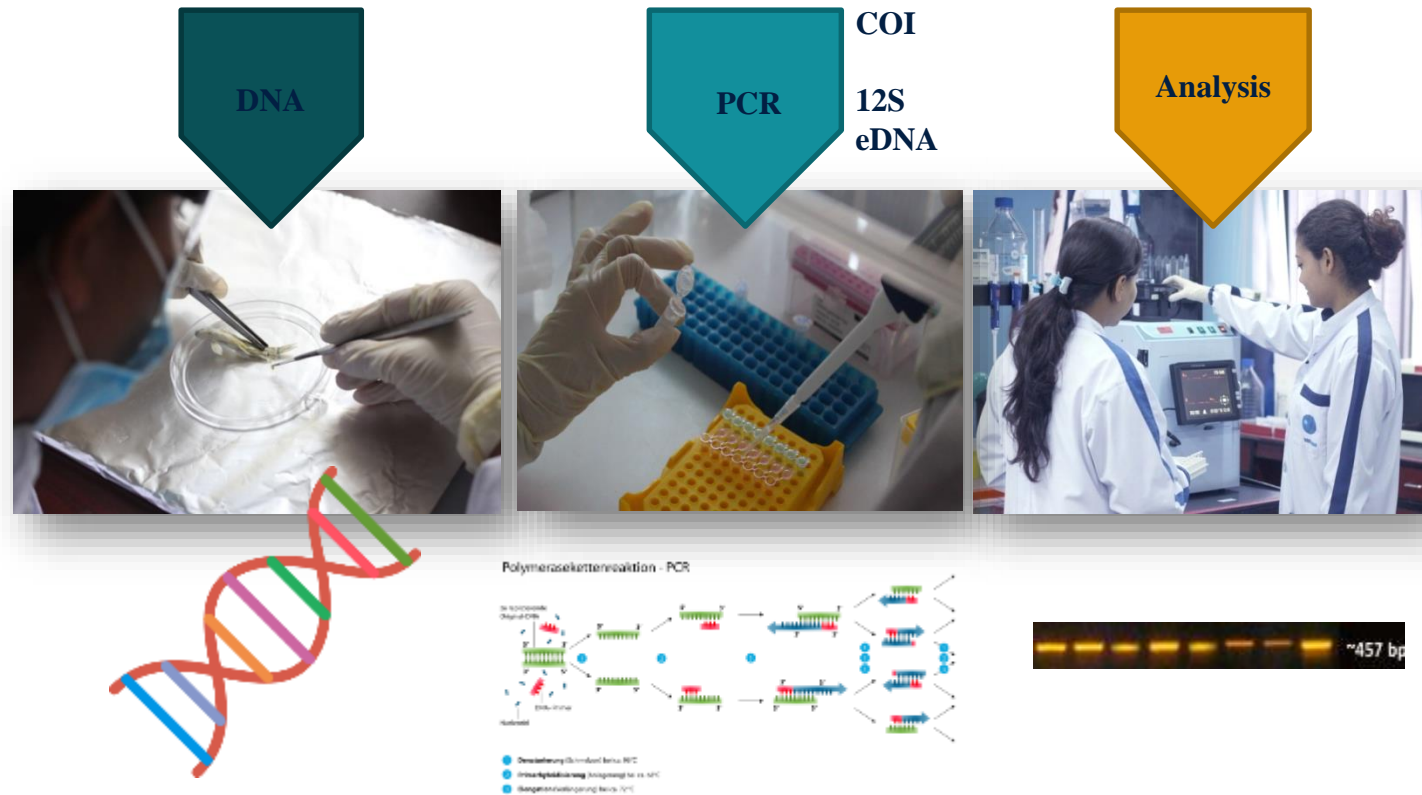




EDNA Sampling in E-Flow Sites		
	River Water-Sample Type	Water Volume (Litre)
1	Upstream	2L
2	Downstream	2L
3	Pool	2L
4	Riffle	2L
5	Sediment	2L
6	Control	1L
	7 E-Flow Sites	6 Samples/Site*7 Sites=42 Samples

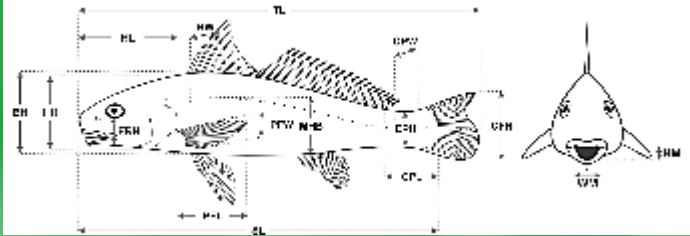


# Laboratory Process

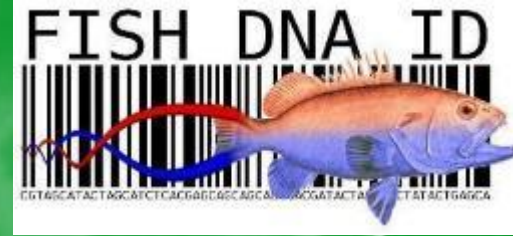




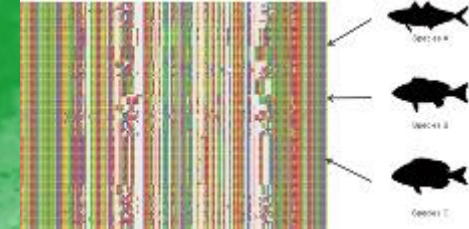
# Fish Identification



Morphological



DNA Barcode (COI)

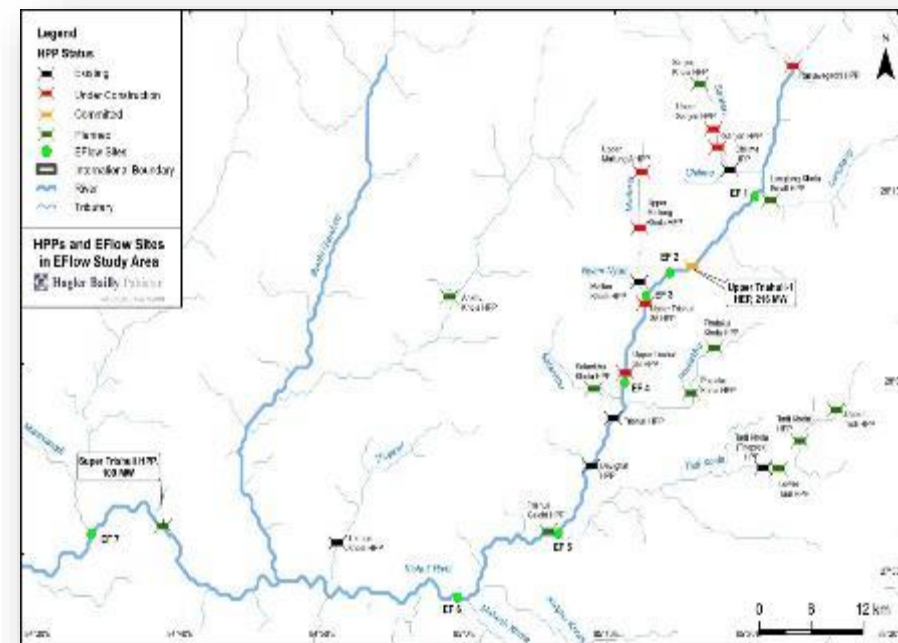


Meta-barcoding (12S)

# Water Chemistry Characteristics

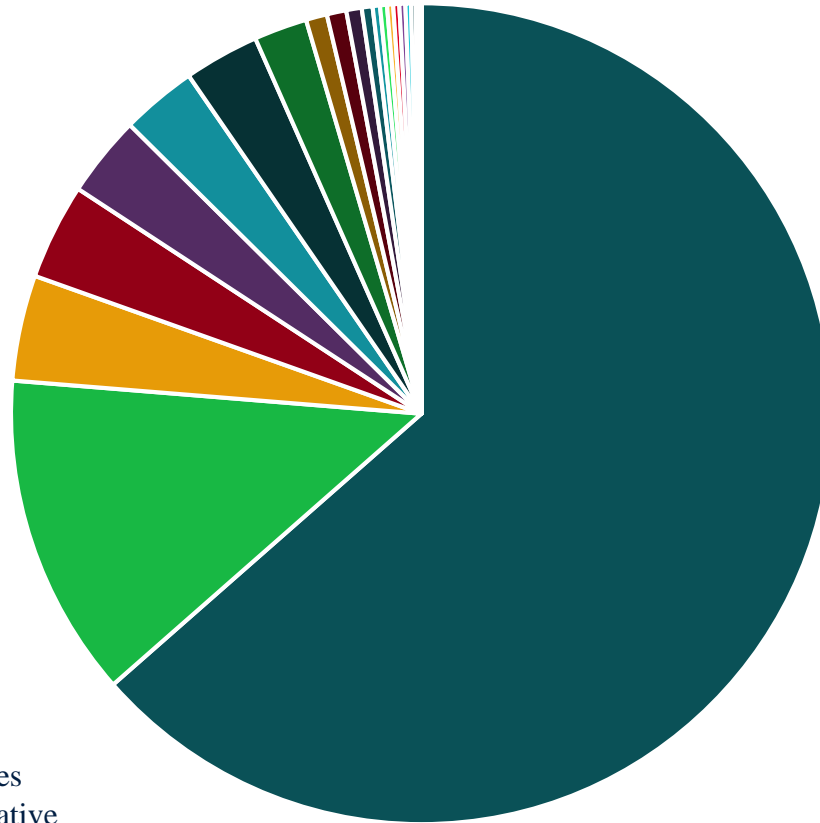


Data Field	EF1	EF2	EF3	EF4	EF5	EF6	EF7
Altitude (m)	1430	1008	860	647	457	386	221
Air Temperature (°C)	19.18	26.3	25.3	26.1	26.1	27.85	25.82
Water temp.(°C)	12.95-12.98	13.49-13.58	13.59-13.8	14.54-15.06	16.43-16.86	20.27-20.42	21.27-22.74
DO (ppm/%)	7.81-8.2	8.33-8.56	8.02-8.84	8.27-8.51	8.1-8.54	7.2-7.7	7.34-7.9
pH	7.74-8.06	8.09-8.36	8.01-8.1	8.02-8.62	7.24-7.32	7.76-8.23	7.82-7.98
Ammonia (mg/L)	0.2	0.07	0.11	0	0	0	0.04
Water depth (cm)	37-79	43-82	29-85	33-79	29-54	27-108	61-88
EC	197-203	185-189	174-191	181-192	187-191	181-185	202-226
TDS (ppm)	97-101	92-95	87-95	92-96	93-96	90-92	104-113
Turbidity (FNU)	46-57.2	55.4-114	63.6-87.2*	54.9-115*	68-73.5	70-75	1000**
Phosphate (mg/L)	2.8	3	2.8	1.4	0.4	1.4	3.3
Nitrate (mg/L)	5.3	0	2.9	4.6	4.3	1	0.4
Hardness (mg/L)	88	86	77	81	77	88	113
COD (mg/L)	2.8	3.2	2	2.4	5.6	2.8	36.8
Iron (mg/L)	1.2	2.16	1.69	0.83	4.44	1.2	47.69
Zinc(mg/L)	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	0.1
Nickel(mg/L)	<0.3	<0.3	<0.3	<0.3	<0.3	<0.3	<0.3
Mercury (mg/L)	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Cadmium(mg/L)	<0.003	<0.003	<0.003	<0.003	<0.003	<0.003	<0.003
Lead(mg/L)	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Arsenic(mg/L)	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005



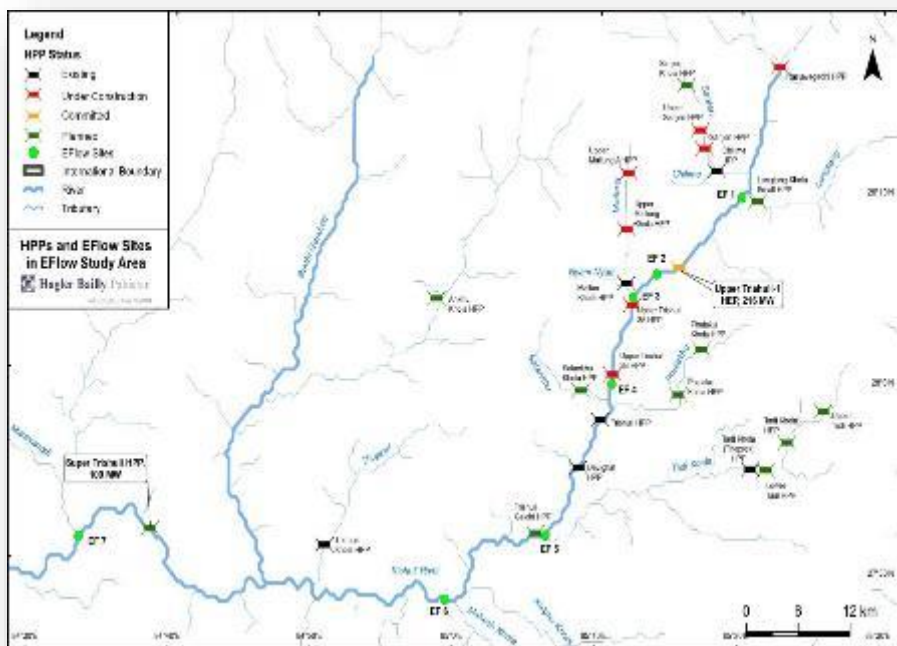


## Species Richness (Relative abundance)

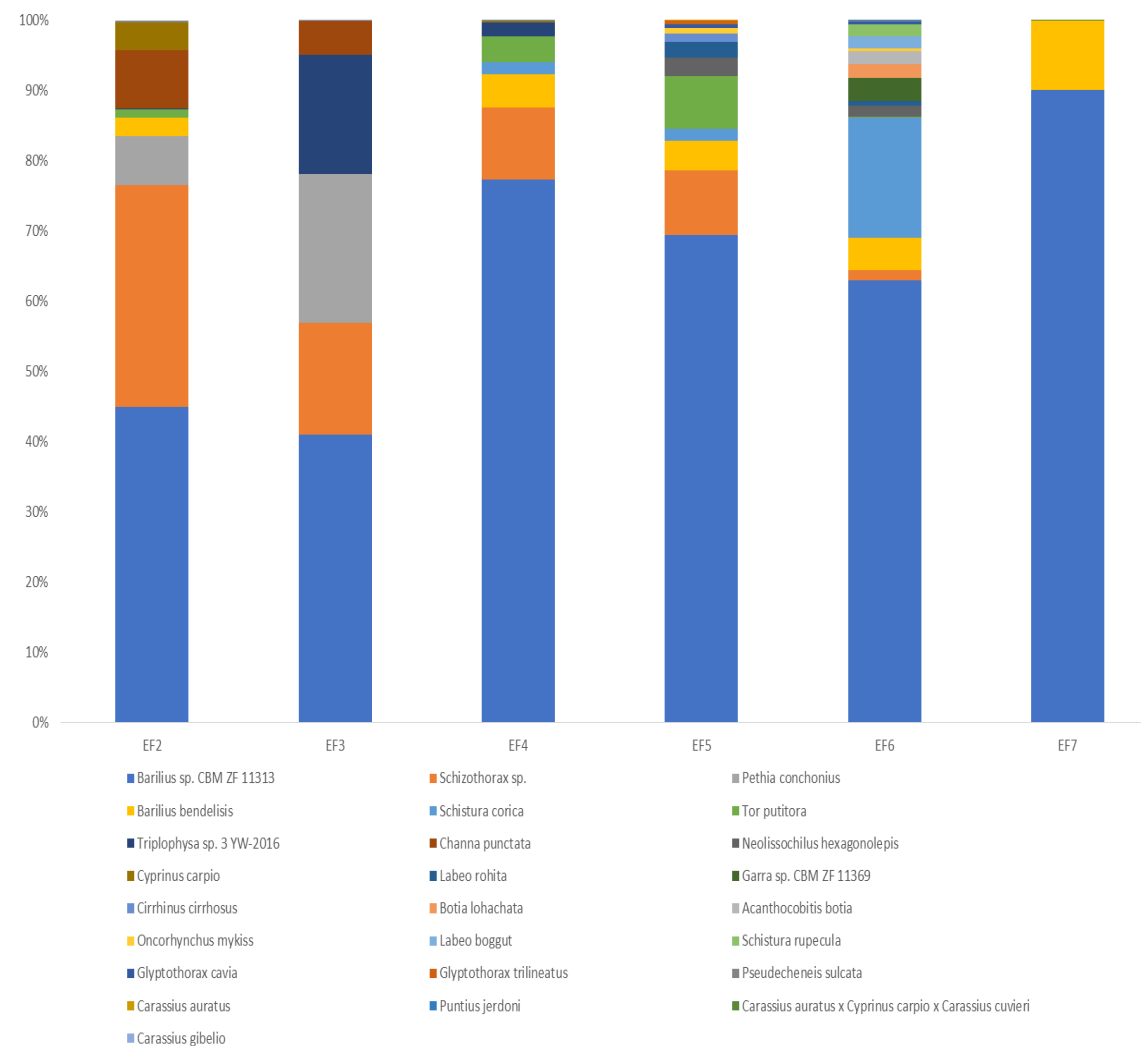


eDNA identified species  
identified and their relative  
abundance (species richness)  
based on eDNA OTU data.

- Barilius sp. CBM ZF 11313
- Schizothorax sp.
- Pethia conchonius
- Barilius bendelisis
- Schistura corica
- Tor putitora
- Triplophysa sp. 3 YW-2016
- Channa punctata
- Neolissochilus hexagonolepis
- Cyprinus carpio
- Labeo rohita
- Garra sp. CBM ZF 11369
- Cirrhinus cirrhosus
- Botia lohachata
- Acanthocobitis botia
- Oncorhynchus mykiss
- Labeo boggut
- Schistura rupecula
- Glyptothorax cavia



## Relative abundance of eDNA identified fish species based on E-Flow sites





SN	Species	Found in Nepal		Found in Trishuli		Indigenous
		Results	Lit. review	Results	Lit. review	
1	<i>Carassius auratus</i>	Yes	Yes	Yes	NA	No
2	<i>Cyprinus carpio</i>	Yes	Yes	Yes	NA	No
3	<i>Oncorhynchus mykiss</i>	Yes	Yes	Yes	Yes	No
4	<i>Puntius/ Pethia conchonius</i>	Yes	Yes	Yes	Maybe	Indigenous
5	<i>Labeo rohita</i>	Yes	Yes	Yes	Yes	Indigenous
6	<i>Carassius gibelio</i>	Yes	No	Yes	No	No
7	<i>Schizothorax sp.</i>	Yes	Yes	Yes	Yes	Indigenous
8	<i>Glyptothorax cavia</i>	Yes	Yes	Yes	Yes	Indigenous
9	<i>Barilius bendelisis</i>	Yes	Yes	Yes	Yes	Indigenous
10	<i>Tor putitora</i>	Yes	Yes	Yes	Yes	Indigenous
11	<i>Carassius auratus x Cyprinus carpio x Carassius cuvieri</i>	Yes	No	Yes	No	No
12	<i>Glyptothorax trilineatus</i>	Yes	Yes	Yes	Yes	Indigenous
13	<i>Channa punctata/punctatus</i>	Yes	Yes	Yes	Maybe	Indigenous
14	<i>Botia lohachata</i>	Yes	Yes	Yes	Yes	Indigenous
15	<i>Neolissochilus hexagonolepis</i>	Yes	Yes	Yes	Yes	Indigenous
16	<i>Cirrhinus cirrhosus/ mrigala</i>	Yes	Yes	Yes	Yes	Indigenous
17	<i>Pseudecheneis sulcata</i>	Yes	Yes	Yes	Yes	Indigenous
18	<i>Acanthocobitis/ Nemacheilus botia</i>	Yes	Yes	Yes	Yes	Indigenous
19	<i>Schistura corica/Nemacheilus corica</i>	Yes	Yes	Yes	Yes	Indigenous
20	<i>Schistura rupecula</i>	Yes	Yes	Yes	Yes	Indigenous
21	<i>Labeo boggut</i>	Yes	No	Yes	No	No
22	<i>Triplophysa sp.</i>	Yes	No	Yes	No	No
23	<i>Barilius sp.</i>	Yes	Yes	Yes	Yes	Indigenous
24	<i>Garra sp.</i>	Yes	Yes	Yes	Yes	Indigenous
25	<i>Puntius jerdoni</i>	Yes	No	Yes	No	No



## eDNA and fish capture

**eDNA Data breakdown**

	Total Count	Common with fish capture	Species level Identification
eDNA	25	6	21

**Fish capture (Genetics) Data breakdown**

	Total Count	Common with eDNA	Species level Identification
Fish	11	6	9

**IUCN Status of eDNA/Fish captured species**

	Species	Not Enlisted	Least Concern	Near Threatened	Vulnerable	Endangered
eDNA	25	7	14	1	2	1
Fish	11	3	6	2	0	0



	eDNA List Phase I	Status	Fish Capture Phase I	Trishuli	EF1	EF2	EF3	EF4	EF5	EF6	EF7	eDNA List Phase II
1	<i>Acanthocobitis/Nemacheilus botia</i>	Least Concern		✓	x	x	x	x	x	✓	x	<i>Acanthocobitis botia</i>
2	<i>Barilius bendelisis</i>	Least Concern	<i>Barilius bendelisis</i>	✓	x	✓	✓	✓	✓	✓	✓	<i>Barilius bendelisis</i>
3	<i>Barilius sp. CBM ZF 11313</i>	Unlisted	<i>Barilius vagra</i>	✓	x	✓	✓	✓	✓	✓	✓	<i>Barilius sp. CBM ZF 11313</i>
4	<i>Botia lohachata</i>	Unlisted	<i>Botia lohachata</i>	✓	x	x	x	x	x	✓	x	<i>Botia lohachata</i>
5	<i>Carassius auratus x Cyprinus carpio x Carassius cuvieri</i>	Least Concern		✓	x	x	x	x	x	x	✓	<i>Channa punctata</i>
6	<i>Carassius auratus/ carassius</i>	Least Concern		✓	x	x	✓	x	✓	✓	✓	<i>Clupisoma garua</i>
7	<i>Carassius gibelio</i>	Unlisted		x	x	x	✓	x	x	x	x	<i>Garra sp. CBM ZF 11369</i>
8	<i>Channa punctata/punctatus</i>	Least Concern		x	x	✓	✓	x	x	x	x	<i>Glyptothorax trilineatus</i>
9	<i>Cirrhinus cirrhosus/ mrigala</i>	Vulnerable		x	x	x	x	✓	✓	x	x	<i>Hypophthalmichthys nobilis</i>
10	<i>Cyprinus carpio</i>	Vulnerable		✓	x	✓	✓	✓	x	✓	x	<i>Labeo bata</i>
11	<i>Garra sp. CBM ZF 11369</i>	Unlisted	<i>Garra sp.</i>	✓	x	x	x	x	x	✓	✓	<i>Labeo boggut</i>
12	<i>Glyptothorax cavia</i>	Least Concern	<i>Glyptothorax garhwali</i>	x	x	x	x	x	✓	✓	x	<i>Neolissochilus hexagonolepis</i>
13	<i>Glyptothorax trilineatus</i>	Least Concern	<i>Glyptothorax garhwali</i>	✓	x	x	x	x	✓	x	✓	<i>Pethia conchoni</i>
14	<i>Labeo boggut/ L. boga(to confirm)</i>	Least Concern			x	x	x	x	✓	✓	x	<i>Puntius chola</i>
15	<i>Labeo rohita</i>	Least Concern		✓	x	x	x	x	✓	✓	✓	<i>Schistura corica</i>
16	<i>Neolissochilus hexagonolepis</i>	Near Threatened	<i>Neolissochilus hexagonolepis</i>	x	x	x	x	✓	✓	✓	✓	<i>Schizothorax sp.</i>
			<i>Neolissochilus hexastichus</i>									<i>Tor putitora</i>
17	<i>Oncorhynchus mykiss</i>	Unlisted		✓	x	x	x	x	✓	✓	x	<i>Tor tor</i>
18	<i>Pethia/ Puntius conchoni</i>	Least Concern		x	x	✓	✓	x	x	x	x	<i>Triplophysa sp. 3 YW-2016</i>
19	<i>Pseudecheneis sulcata</i>	Least Concern		✓	x	✓	x	x	x	✓	x	
20	<i>Puntius jerdoni</i>	Least Concern		x	x	x	✓	x	x	✓	x	
21	<i>Schistura corica/Nemacheilus corica</i>	Least Concern	<i>Schistura corica</i>	✓	x	x	x	✓	✓	✓	✓	
22	<i>Schistura rupecula</i>	Least Concern		x	x	x	x	x	x	✓	x	
23	<i>Schizothorax sp.</i>	unlisted	<i>Schizothorax sp.</i>	✓	x	✓	✓	✓	✓	✓	✓	
24	<i>Tor putitora</i>	Endangered		✓	x	✓	x	✓	✓	✓	✓	
25	<i>Triplophysa sp. 3 YW-2016</i>	Unlisted		✓	x	✓	✓	✓	✓	x	x	
			<i>Opsarius cf. shacra</i>									
			<i>Crossocheilus latius</i>									

# Standardized Sampling of Aquatic Organisms to Monitor Population Trends in Nepal: Environmental DNA

Feb-March Dry season 2020

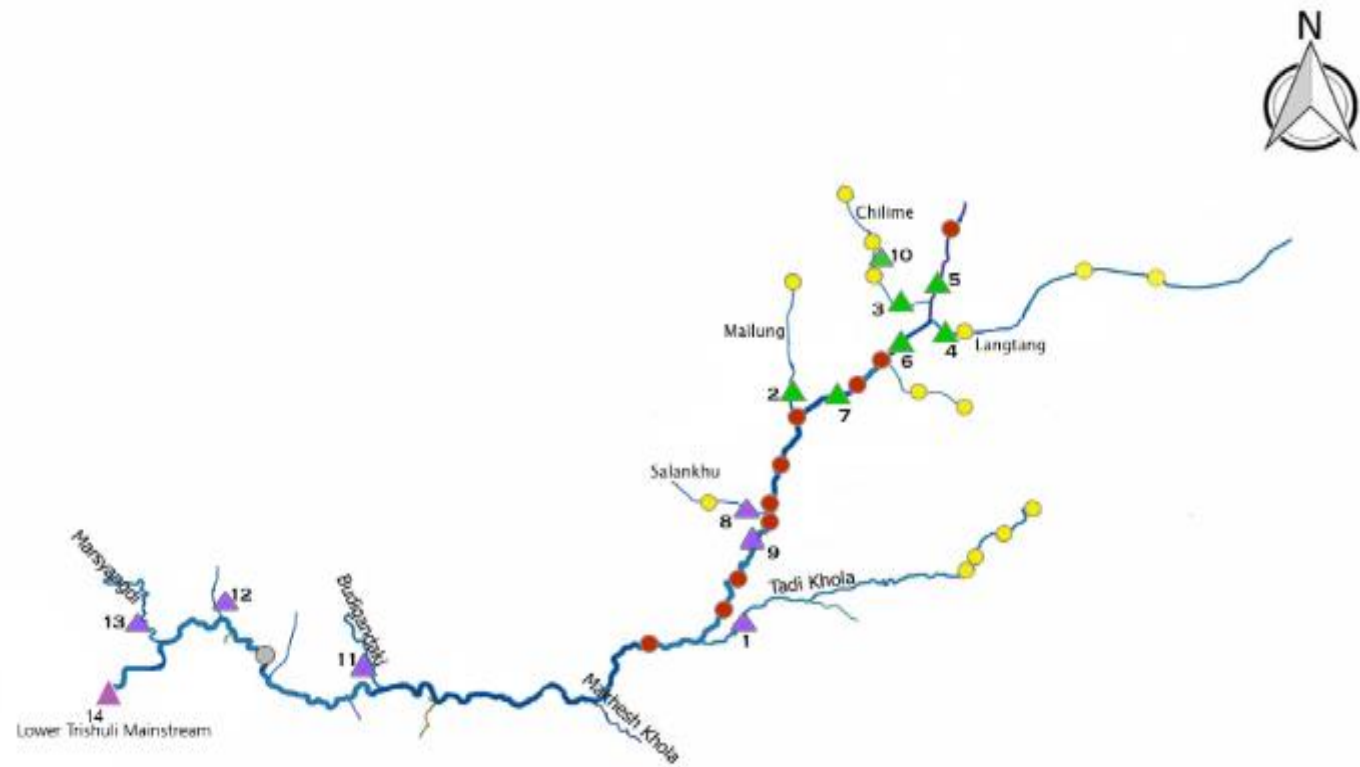




This field study was conducted as part of the Trishuli Tools Assessment Survey which explored multiple fish collection techniques in a comparative effort to explore the best practice approach and sampling standardization of fish monitoring trends in Nepal. A major hurdle for aquatic monitoring in Nepal particularly fish monitoring has to do with inconsistencies in fish collection methodologies resulting in incomplete or incomparable baseline databases due to variations in collection standards (sampling type, catch per unit efforts among others). Each fish sampling technique is suited to custom topographies some suiting better to one than the other, all that holding their merits and demerits.



# Study Sites



**FIGURE LEGEND**

- Hydropower Dam – Main Channel of the Trishuli River
- Hydropower Dam – Tributary of the Trishuli River
- Hydropower Dam – Planned
- ▲ Sampling site with Site Number – Location within Langtang National Park
- ▲ Sampling site with Site Number – Location outside Langtang National Park

**SAMPLING SITES**

- 1. Tadi Khola
- 2. Mailung Khol
- 3. Lower Chilime
- 4. Langtang Khola
- 5. Upper Bhote Kosi
- 6. Upper Trishuli River
- 7. UT-3 Dewatered Zone
- 8. Salankhu Khola
- 9. Below Trishuli Dam
- 10.Upper Chilime (No eDNA sample)
- 11.Budi Gandhaki Nadi
- 12.Juddhi Khola (No eDNA sample)
- 13. Marsyangdi Nadi
- 14. Lower Trishuli Mainstream



# 16 Taxa Hits

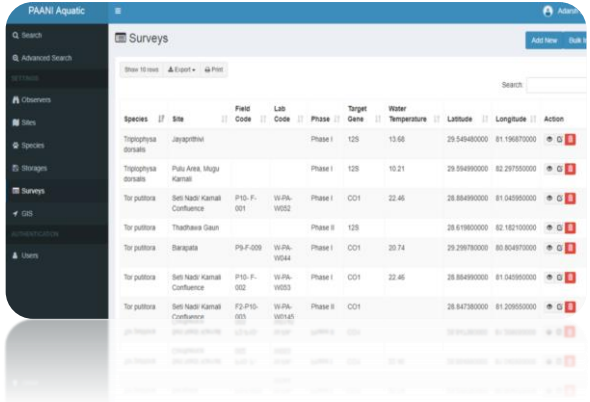


	staxids	sscnames	EF-10	EF-6	EF-1	EF-3	EF-4	EF-2	EF-17	EF-9	EF-11	EF-14	EF-15	EF-16	
SN	NCBI Taxa ID	Scientific Name	Tadi Khola	Mailung	Lower Chilime Khola	Langtang Khola	Bhote Koshi	Trisuli Mainstream Above	Salekhu	Below Trishuli Dam	Budi Gandaki Nadi	Judhi Khola	Lower Trishuli Mainstream #2	Marsyangdi	Total
			TAD	MAI	LCH	LAN	UBK	UTR	SAK	BTD	BUD	JUD		MAR	
		Total	32741	19389	65641	7	15770	46985	37873	51326	24264	7033	3	48558	349590
1	183907	<i>Schizothorax spp.</i>	8097	19121	50268	7	15769	46984	21895	46908	4064	190	1	526	213830
2	596149	<i>Schistura corica</i>	13232		1	0	0	0	1	0	433	0	1	37213	50881
3	322109	<i>Botia lohachata</i>	2	0	0	0	0	1	1	0	16505	0	0	1	16510
4	231675	<i>Glyptothorax trilineatus</i>	0	0	15337	0	0	0	0		0	0	0	0	15337
5	210633	<i>Puntius jerdoni</i>	2949	0	1	0	0	0	11122	0	132	0	0	0	14204
6	209118	<i>Barilius bendelisis</i>	1880	0	2	0	0	0	4852	688	81	5698	0	0	13201
7	643451	<i>Chagunius chagunio</i>	3	36	30	0	0	0	0	414	1	1	1	10817	11303
8	370357	<i>Neolissochilus hexagonolepis</i>	4228	48	0	0	0	0	2	1063	91	0	0	0	5432
9	643349	<i>Schistura rupecula</i>	0	0	0	0	1	0	0	275	2110	0	0	0	2386
10	1913164	<i>Garra sp. CBM ZF 11369</i>	1624	0	0	0	0	0	0	0	0	595	0	1	2220
11	1569704	<i>Oreochromis niloticus x Oreochromis</i>	0	1	0	0	0	0	0	1344	0	0	0	0	1345
12	425510	<i>Acanthocobitis botia</i>	726	0	0	0	0	0	0	0	0	549	0	0	1275
13	1913148	<i>Barilius sp. CBM ZF 11313</i>	0	114	0	0	0	0	0	617	0	0	0	0	731
14	932675	<i>Labeo boggut</i>	0	0	1	0	0	0	0	0	445	0	0	0	446
15	199216	<i>Glyptothorax cavia</i>	0	0	1	0	0	0	0	0	402	0	0	0	403
16	385307	<i>Pseudecheneis sulcata</i>	0	0	0	0	0	0	0	17	0	0	0	0	17



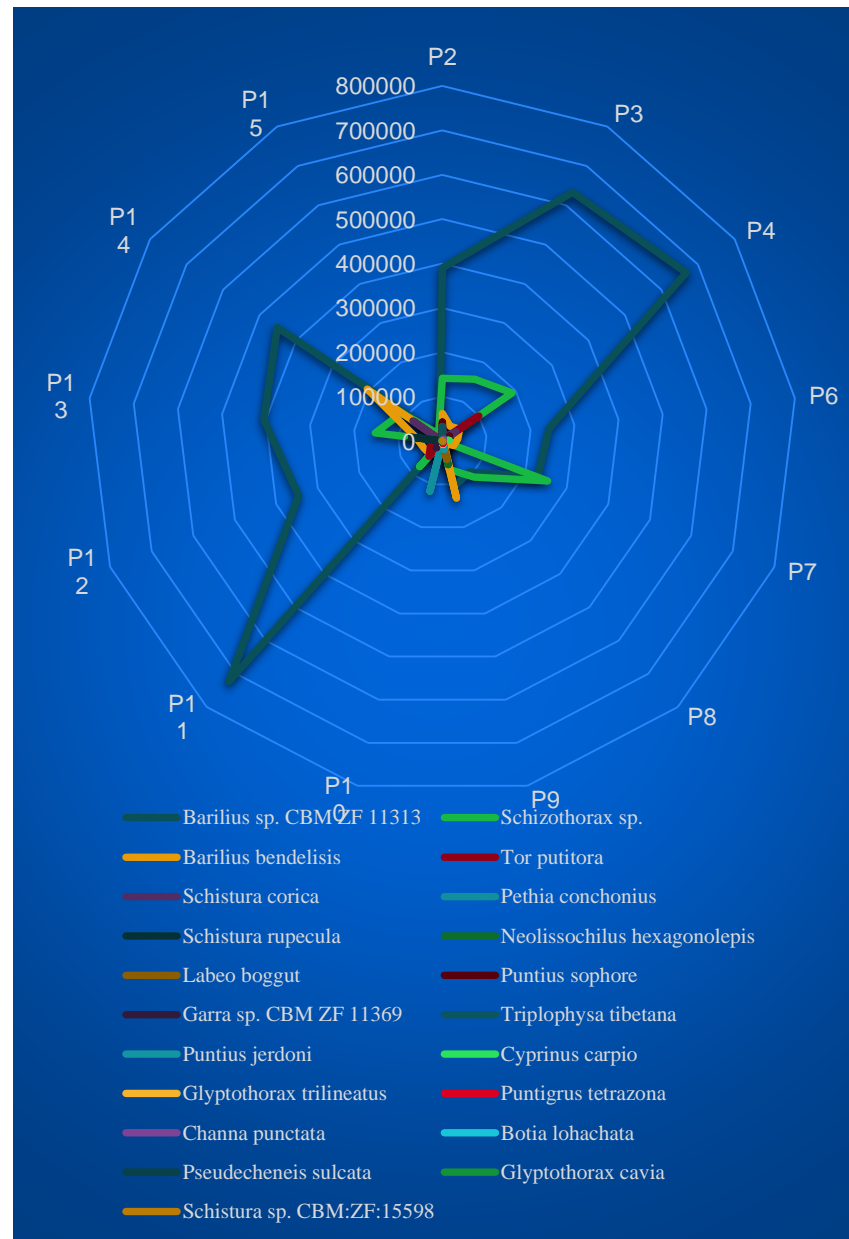
SN	Capture Fish (Generic Field Identified Morphological identification only)	eDNA identified species
1	<i>Schizothorax richardsonii</i>	<i>Schizothorax</i> spp.
2	<i>Pseudecheneis sulcatus</i>	<i>Oreochromis niloticus</i> x <i>Oreochromis aureus</i>
3	<i>Garra annandalei</i>	<i>Schistura corica</i>
4	<i>Garra gotyla</i>	<i>Botia lohachata</i>
5	<i>Barilius barila</i>	<i>Neolissochilus hexagonolepis</i>
6	<i>Barilius bendelisis</i>	<i>Garra</i> sp. CBM ZF 11369
7	<i>Paracanthocobitis botia</i>	<i>Puntius jerdoni</i>
8	<i>Pethia conchoni</i>	<i>Barilius bendelisis</i>
9	<i>Channa gachua</i>	<i>Acanthocobitis botia</i>
10	<i>Neolissochilus hexagonolepis</i>	<i>Glyptothorax trilineatus</i>
11	<i>Naziritor chelynoides</i>	<i>Barilius</i> sp. CBM ZF 11313
12	<i>Schistura corica</i>	<i>Pseudecheneis sulcata</i>
13	<i>Physoschistura elongate</i>	<i>Chagunius chagunio</i>
14	<i>Glyptosternum blythi</i>	<i>Schistura rupecula</i>
15	<i>Noeimacheilus rupicola</i>	<i>Glyptothorax cavia</i>
16	<i>Pethia</i> spp	<i>Labeo boggut</i>
17	<i>Channa</i> spp	
19	<i>Glyptothorax</i> spp.	



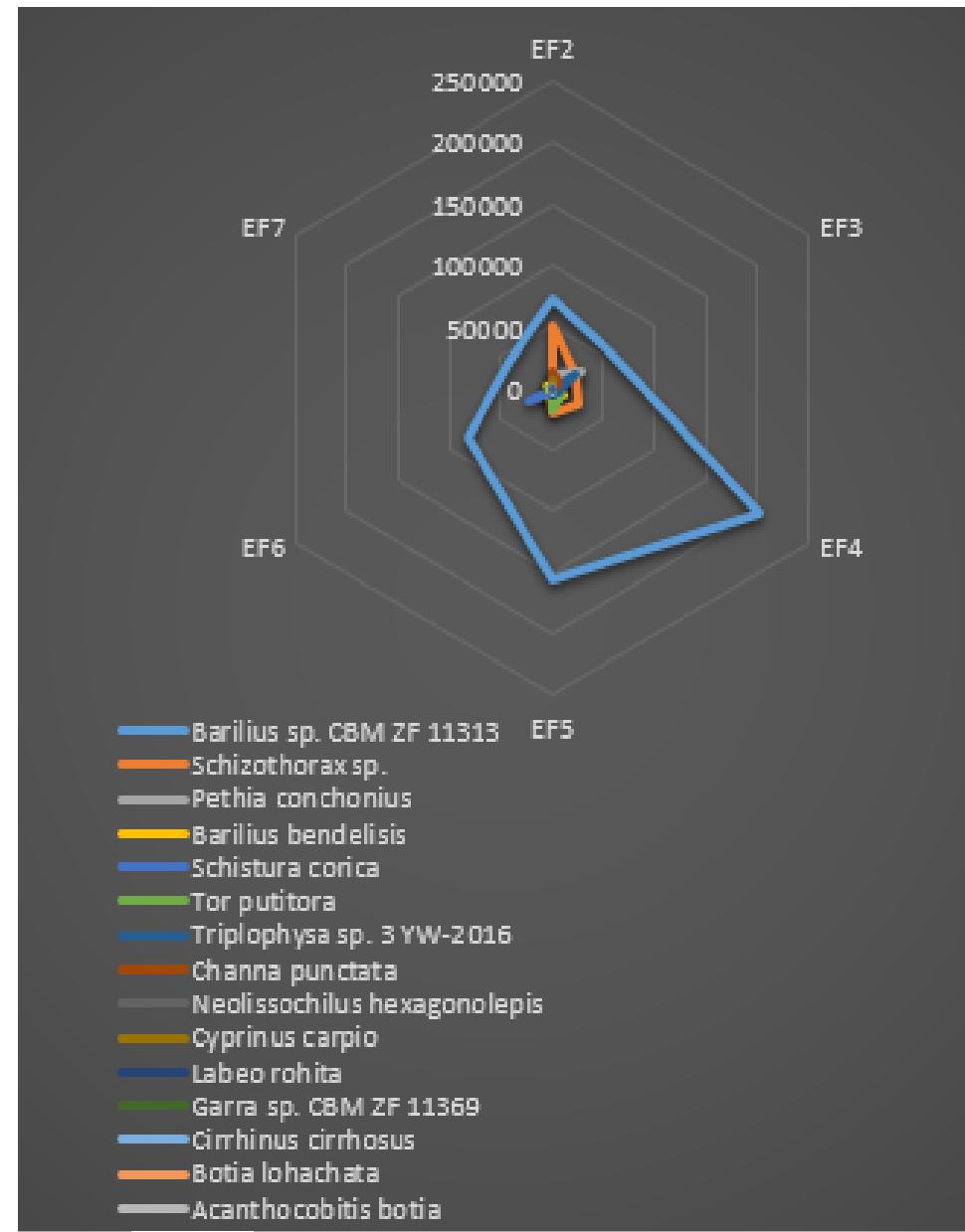


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## Fish eDNA abundance KARNALI RIVER



## Fish eDNA abundance TRISULI RIVER





## Moving Forward

Increased/frequent sampling

Tributary sampling

Direct field based kits



## Handheld DNA Device



