

POST-MONSOON COMMUNITY STRUCTURE OF FISHERIES IN THE NIKACHHU RIVER AT THE NIKACHHU HYDROPOWER PROJECT AREA: AN ATTEMPT TO STANDARDIZE AND IMPROVE HYDROPOWER IMPACT ASSESSMENT APPROACHES IN BHUTAN

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Abstract: Realizing the expanding nature of hydropower developments and their consequences on aquatic biodiversity, hydropower regulators and promoters in Bhutan are mandated to address impacts through the implementation of conservation and management plans devised through regular monitoring. This study presents findings from post-monsoon monitoring (October – November 2021) conducted at Nikachhu within the vicinity of the Nikachhu Hydropower Project. A paired *t*-test (at $p = 0.05$) conducted during the advanced stage of construction-phase indicated no significant differences among the assessed biotic and abiotic variables with exception of temperature ($p = 0.023$) between the upstream (control) and downstream (impacted) stretches of Nikachhu. The upstream stretch was dominated by *Salmo trutta*, whereas *Schizothorax richardsonii* dominated the downstream stretches. The exclusive occurrences of glyptosternids were reported from the upstream (*Creteuchiloglanis* sp.) and downstream stretches (*Parachiloglanis* sp.). The species richness (*S*), abundance (*n*) and catch per unit effort (CPUE) decreased from the upstream to the dam construction site and gradually increased thereafter. The highest catch per unit effort and abundance recorded from sites away from anthropogenic influence and natural extremities suggest the combined influence of these factors on fishes. However, the lack of standardized sampling data from earlier monitoring makes spatio-temporal impact assessment unreliable. Hence, the implementation of adaptive approach-based standardized long-term monitoring (qualitative and quantitative) should be considered for the holistic realization of conservation actions related to future hydropower developments in Bhutan.

Keywords: Catch per unit effort; monitoring; population structure

1. INTRODUCTION

Rivers and streams are vital for sustaining ecosystem processes and fostering socio-economic development. They have contributed food to humans and provided water for household and agricultural consumption, inland transportation and hydropower generation (Grill et al. 2019). Of recent, Bhutanese rivers are increasingly regulated to harness 26,760 MW of estimated techno-economically feasible hydropower potential (NORAD 2017). The total installed energy

production capacity from hydropower projects (HPPs), as of 2021 is 2344 megawatt (MW): 2,326 MW from 6 major HPPs (> 25 MW) and 16 MW from 24 small and mini/micro HPPs (< 25 MW) (NORAD 2017). To harness the enormous hydropower potential, all major rivers and their tributaries are earmarked for hydropower development, several projects are planned, and some are prioritized. Presently, the major HPPs which are under various stages of development are the 1200 MW Punatsangchhu – I Hydropower Project (PHPP – I), 1020 MW Punatsangchhu – II

Hydropower Project (PHPP – II), 600 MW Kholongchhu Hydropower Project (KHPP) and 118 MW Nikachhu Hydropower Project (NHPP).

The human-induced pressure from water infrastructure development such as dams is associated with subsequent pressures from river fragmentation, sediment trapping, water consumption and flow regulation, affecting the normal functions of healthy rivers (Grill et al. 2019). Depending on the size of the infrastructure, the construction phase is associated with changes in morphological characteristics (e.g., from the extraction of boulder, gravel, sand and substrate homogenization by washed off excavated debris), early changes in limnological conditions (e.g., from the discharge of effluents and sediments) and initial fragmentation of longitudinal connectivity of river (Jozi and Maryam 2014; Jozi et al. 2015; Rezaian et al. 2016). Upon commissioning, the transformation of the river into a reservoir is characterized by changes in hydro-morphological conditions such as flow reduction, stagnation, sedimentation and substrate homogenization (Schmutz and Moog 2018), and limnological changes such as thermal stratification, acidification, eutrophication, and anoxia (Santos et al. 2017; Schmutz and Moog 2018) cascading the impact on aquatic biodiversity (Li et al. 2013; Santos et al. 2017; Schmutz and Moog 2018).

The dam acts as a barrier obstructing the downstream transport regime of sediments and nutrients, reducing the biological productivity of floodplains and in areas of high fisheries productivity such as the Lower Mekong Basin, dam construction resulted in a higher loss of biodiversity than the climate change (Yoshida et al. 2020). The fragmentation of rivers by dams results in upstream-downstream connectivity loss, denying bidirectional access of fishes to spawning, growing and feeding habitats (Zhang et al. 2018; Moreno-Arias et al. 2021). Consequently, 76.2 % of migratory and 41.9 % of non-migratory large freshwater fishes are threatened by dam constructions (He et al. 2021) and the same is expected for numerous small-sized fishes. The downstream flow regulation alters thermal and oxygen regimes along a longitudinal continuum across spatio-temporal scale (Meier et al. 2003; Dickson et al. 2012; Jumani et al. 2018; Chandesris

et al. 2019). It also modifies hydromorphological conditions, reducing suitable spawning habitats along dewatered stretches and the change in thermal regimes alters spawning periods of endemic fishes (Zhang et al. 2018). These changes will have adverse impacts on migration (Santos et al. 2017; He et al. 2021), spawning, survival and growth (Humphries et al. 2002; He et al. 2021), recruitment (Humphries et al. 2002; Joshi et al. 2014; Lima et al. 2017) and diversity, distribution and composition of fishes (Li et al. 2013; Joshi et al. 2014; Song et al. 2016; Santos et al. 2017; Jumani et al. 2018).

The severity of changes in ecological processes and subsequent consequences on aquatic biodiversity increases with multiple dams within a river system favouring invasion by exotic fishes (often upwards of reservoirs), resulting in rapid declines of native fishes (Li et al. 2013; Santos et al. 2017), altering their functional structure (Li et al. 2013; Smith et al. 2017; Arantes et al. 2019). Therefore, with a worldwide increase in the development of small and large HPPs (Zarfl et al. 2015; Couto and Olen 2018), hydropower dams are among the major threat to the global aquatic biodiversity and need immediate attention (Dudgeon et al. 2006; Reid et al. 2019).

In Bhutan, considering the vulnerability of aquatic ecosystems to dam constructions, the Water Regulation of Bhutan (2014) requires hydropower developers to address adverse environmental impacts through conservation and mitigation measures such as (i) release of scientifically determined environmental flows (e-flows), and (ii) operation of fish migration structures to facilitate bi-directional migration of fish, or if not feasible, on-site hatcheries should be operated for stock replenishment programs. Therefore, an adequate information on the spatio-temporal structure of fisheries communities, both before and after commencement of hydropower operations obtained through regular monitoring of the rivers/stream within the project area is necessary to develop effective conservation and mitigation measures. The long-term information on catch per unit effort (CPUE), abundance (n), species richness (S) and assemblage structures of fisheries in the monitoring sites provide a good indication of the impact of hydropower operation on fisheries

composition (Li et al. 2013; Santos et al. 2017; Santos et al. 2022). However, earlier bi-annual monitoring (2016 – 2020) conducted at Nikachhu, where 118 MW Nikachhu HPP is currently at an advanced stage of construction was focused on presence-absence and length data with inconsistent sampling effort across seasons. The present post-monsoon monitoring is therefore aimed to access and compare: (i) basic water quality parameters and their patterns, and (ii) the composition structures of fisheries and biotic indices such as the CPUE, n and S between the upstream (i.e., control, unimpacted) and downstream (i.e., impacted) stretches of Nikachhu HPP area. The overall goal of the study was to make a preliminary attempt to standardize and improve fish-based approaches used in monitoring the impacts of hydropower developments on aquatic biodiversity in Bhutan.

2. MATERIALS AND METHODS

2.1 Study area

The study was conducted in Central Bhutan, mainly along the Nikachhu at Trongsa (two sites

are at Wangdue Phodrang) with the inclusion of some stretches of Mangdechhu (≈ 42.70 km river length) at Trongsa (Figure 1, Table 1). The Nikachhu originates from Wangdue Phodrang and drains into Mangdechhu below Tangsibji, Trongsa. The 118 MW Nikachhu HPP is currently under advanced stages of construction at Nikachhu, while the 720 MW Mangdechhu HPP is operational at Mangdechhu. Upon completion, the water from the tailrace tunnel of Nikachhu HPP will drain into the dam of Mangdechhu HPP to achieve additional power generation. The Nikachhu stretch (≈ 22.900 km river length from Chazam Archery Ground: Site 1, Figure 1, Table 1 till the Nikachhu-Mangdechhu confluence: Site 12, Figure 1, Table) were divided into upstream (≈ 12.500 km from Site 1 to Site 6) and downstream stretch (≈ 10.400 km from Site 7 to Site 12). The Mangdechhu stretch (≈ 19.800 km river length) includes dewatered stretch downstream of Mangdechhu Dam (Site 13, Figure 1, Table 1) till the powerhouse of diversion-type run-of-the-river Mangdechhu HPP (Site 15 and 16, Figure 1, Table 1). The dominant activity across the study area is agricultural farming (paddy and vegetables).

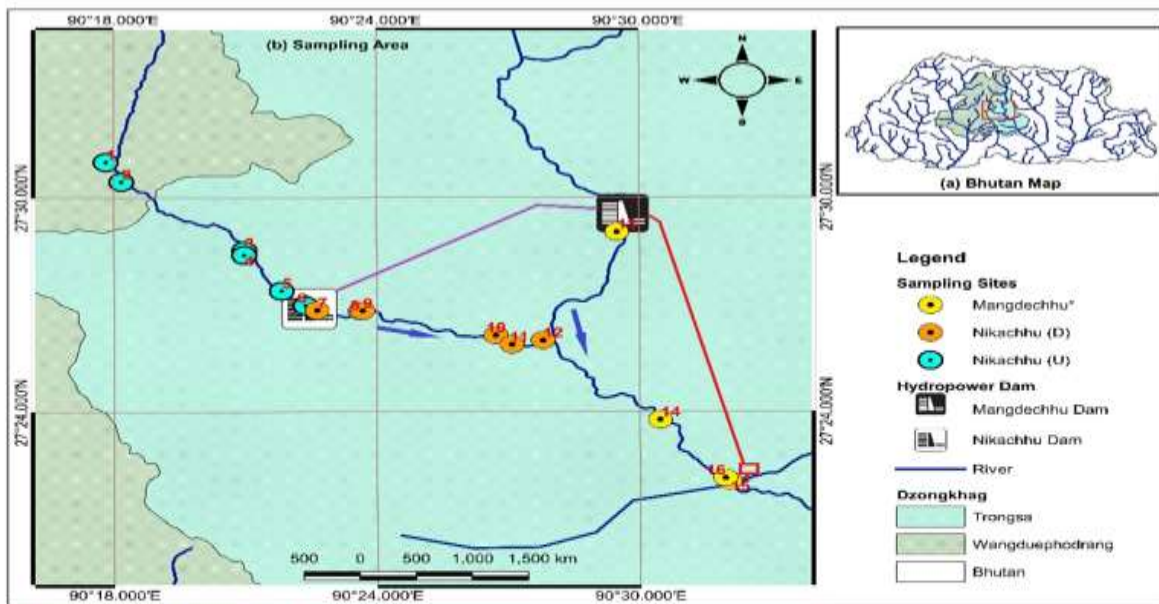


Figure 1: Location of the study area and sampling sites: (a) map of Bhutan showing the study area (red square), and (b) the location of sampling sites along the monitoring area (Nikachhu River and some stretches of Mangdechhu River; refer to table 1 for details). Nikachhu (U) = upstream of the Nikachhu Dam (under advanced stage of construction), Nikachhu (D) = downstream of the Nikachhu Dam (under advanced stage of construction), Mangdechhu* refers downstream of Mangdechhu from Mangdechhu Dam (operational stage) including its sampled tributaries, i.e., Wochenchhu. Sites numbers (i.e., serial #) are from upstream to the downstream end. **Additional legends (Figure 1b):** blue arrow = direction of river flow, purple line = water conveyance system of Nikachhu HPP (approximated), red line = water conveyance system of Mangdechhu HPP (approximated), red square = powerhouse of Mangdechhu HPP (approximated).

2.2 Sampling design

A total of 16 sampling sites (Figure 1, Table 1) were established along the entire study area with an altitudinal gradient of 1,369 m, of which 12 sites lie along the Nikachhu (altitudinal gradient of 1,111m; 6 upstream sites, i.e., control or unimpacted sites by hydropower construction, in particular, i.e., Site 1 to 6, Figure 1, Table 1 and 6 downstream sites, i.e., impacted sites, i.e., Site 7 to 12, Figure 1, Table 1) and 4 sites along the Mangdechhu dewatered section (altitudinal gradient \approx 490 m, i.e., Site 13 to 16, Figure 1, Table 1). The sampling sites along Nikachhu range from 16 – 492 m changes in elevation as the inaccessible geographical terrain made it impossible to establish sites with approximately uniform altitudinal drop. The sampled tributaries include the Chendebjichhu, a small stream draining to Nikachhu (upstream of Nikachhu Dam) and Wochenchhu, a small stream joining the Mangdechhu at dewatered reach near the powerhouse of MHPP. The Mangdechhu stretch was included only to ascertain the diversity of

fishes along the elevational gradient in the study area (i.e., simple linear regression analysis) and thus the main focus of comparison of this study is mainly between the upstream and downstream stretches of Nikachhu.

2.3 Sample collection

The sampling was conducted during the post-monsoon season (25th October to 3rd November 2021). Sites with multi-habitat conditions (pools, riffles, run and wooden debris) were considered for sampling. Fish samples were collected using an electrofisher (Model: ELT62-2D; Grassl, Germany; DC 3KV) with 20 minutes of sampling effort across all the sites. The fishes were released after taking total and standard length (accuracy of \pm mm) and weight (Model: PHSO40, Pesola, China) for larger samples (as the weighing balance was not suitable to take measurements below 10 g). Taxa that could not be identified at the field were collected, fixed with 10% formalin, and preserved in 70% ethanol for identification and future reference. These taxa were identified either

Table 1: Details of sampling sites at Nikachhu and Mangdechhu near the 118 MW Nikachhu HPP area, Trongsa.

Site. No.	River	Stretch	Date	Latitude (DD)	Longitude (DD)	Altitude (m)
1	Nikachhu	Nikachhu (U)	30-10-21	27.516558	90.296902	2446
2	Nikachhu	Nikachhu (U)	30-10-21	27.507386	90.302841	2423
3	Chendebjichhu	Nikachhu (U)	30-10-21	27.475416	90.349637	2286
4	Nikachhu	Nikachhu (U)	30-10-21	27.473180	90.349547	2268
5	Nikachhu	Nikachhu (U)	30-10-21	27.456449	90.363799	2177
6	Nikachhu	Nikachhu (U)	29-10-21	27.449587	90.372889	2149
7	Nikachhu	Nikachhu (D)	29-10-21	27.447415	90.377301	2133
8	Nikachhu	Nikachhu (D)	29-10-21	27.447104	90.393658	2044
9	Nikachhu	Nikachhu (D)	29-10-21	27.447228	90.394615	2030
10	Nikachhu	Nikachhu (D)	27-10-21	27.435784	90.445260	1538
11	Nikachhu	Nikachhu (D)	26-10-21	27.431448	90.451427	1449
12	Nikachhu	Nikachhu (D)	25-10-21	27.433380	90.463205	1335
13	Mangdechhu	Mangdechhu*	01-11-21	27.483891	90.491421	1567
14	Mangdechhu	Mangdechhu*	04-11-21	27.396317	90.507693	1154
15	Mangdechhu	Mangdechhu*	04-11-21	27.367676	90.534063	1068
16	Wochenchhu	Mangdechhu*	04-11-21	27.369084	90.532484	1077

Note: Nikachhu (U) = upstream of Nikachhu Dam, Nikachhu (D) = Downstream of Nikachhu Dam, Mangdechhu* refers downstream of Mangdechhu Dam including its sampled tributaries, i.e. Wochenchhu.

at the species or genus level at the National Research and Development Centre for Riverine and Lake Fisheries, Haa using the available taxonomic keys (Viswanath et al. 2007; Zhou et al. 2011; Thoni and Gurung 2018). Triplicates of basic water quality parameters such as dissolved oxygen (DO), pH, temperature and total dissolved solids (TDS) were recorded from all the sites with a digital multi-parameter probe (MODEL: 98194; Hanna Instruments, Romania).

2.4 Data analysis

A length-weight relationship was used to determine the weight of *Salmo trutta* (Tanir and Fakioğlu 2017) and *Schizothorax richardsonii* (Tyagi et al. 2014) as (i) the weighing machine used was not suitable for taking smaller measurements, (ii) earlier collected data lacked information on weight for most of the sampling periods and (iii) the relationship derived from available information (April and November 2016) was deemed unclear. The catch per unit effort (CPUE expressed as grams per 20 minutes of electrofishing), abundance (number of fishes, n expressed per 20 minutes of electrofishing), and species richness (S expressed per 20 minutes of electrofishing) were the main biotic variables compared between the upstream and downstream of Nikachhu. Outliers were retained during analysis carefully considering sites/locations where outliers were encountered and also by taking into account limited samples ($n = 12$; 6 = upstream; 6 = downstream) and the idiosyncratic nature of biological and environmental data. After subjecting the biotic and abiotic variables to the requirements of a statistical test (normality test and homogeneity of variance test), a parametric t -test (T-test, $p = 0.05$) for performed for species richness (S) and a non-parametric paired-sample t -test (Wilcoxon, $p = 0.05$) was performed for other biotic variables (CPUE and n) and all the abiotic variables to ascertain the differences between the upstream and downstream of Nikachhu. The relationship between species richness and altitude was evaluated through simple linear regression ($p = 0.05$). The difference in upstream and downstream: (i) diversity of fishes was visualized through a Venn diagram, (ii) species composition by abundance and weight were determined by a heatmap, (iii) population structure of dominant

species was obtained through a histogram and (iii) abundance composition by ecological status (native vs exotic), feeding preferences and flow preferences were depicted through a stacked bar graph. The overall Shannon diversity index (H) and evenness (J) for upstream and downstream stretches were determined. All the analyses were performed in R statistical software.

3. RESULTS AND DISCUSSION

3.1 Basic water quality parameters

Optimum water quality conditions are necessary to support diverse communities of aquatic organisms. In terms of basic water quality parameters, pH and TDS were higher across the upstream, while DO and temperature were higher across the downstream of Nikachhu. However, with exception of temperature (Figure 2c, Table 2), the differences observed for other physiochemical parameters between the upstream and downstream stretches were insignificant (Figure 2a, 2b and 2d, Table 2). The general pattern of all the water quality parameters lacked clear trends along the longitudinal continuum (Supplementary graphs: S1a, S1b, S1c and S1d).

The observed DO (Figure 2a, Table 2) and pH (Figure 2b, Table 2) were within the Indian water quality standards for the propagation of wildlife and fisheries (CPCB 2007) (Class D; DO: ≥ 4 ppm; pH: 6.5 – 8.5) and USEPA's (United States Environment Protection Agency) Causal Analysis/Diagnosis Decision Information System (CADDIS) for freshwater (USEPA 2017a; USEPA 2017b) (DO: 5 ppm; pH: 6.5 – 9). Albaster and Lloyd (1982) suggested a minimum DO concentration of 5 ppm to satisfactorily support the life stages of most aquatic fauna. Successful captive breeding of hill stream fishes with high survival rates was demonstrated in Arunachal Pradesh, India at DO > 6.5 ppm (Abujam et al. 2017). Therefore, the observed DO along the longitudinal continuum (Supplementary graph: S1a; 7.8 – 8.69) is supposed satisfactory for the propagation of fishes in the wild. The pH of water influences the physiological and metabolic function of aquatic organisms affecting embryo development, reproductive performance, survival and growth (Swain et al. 2020). Although pH was

slightly above the CPCB standards in some sites (Supplementary graph: S1b: Site 1: 8.53 and Site 2: 8.73;), the pH range of 6 – 9 is considered satisfactory on a long-term basis for aquatic

Table 2: Comparison of mean, standard deviation (SD) and paired-sample *t*-test results ($p = 0.05$) of biotic variables and abiotic variables between upstream [Nikachhu (U)] and downstream [Nikachhu (D)] of Nikachhu at 118 MW Nikachhu HPP site, Trongsa.

Abiotic variables	Mean \pm SD		test statistic	<i>p</i> -value
	Nikachhu (U)	Nikachhu (D)		
DO (ppm)	8.37 \pm 0.25	8.41 \pm 0.29	103	0.218
pH	8.32 \pm 0.26	8.28 \pm 0.25	49	0.201
Temperature ($^{\circ}$ C)	9.74 \pm 0.83	10.76 \pm 1.57	138	0.023
TDS (ppm)	37.50 \pm 15.97	34.17 \pm 2.15	46	0.265
EC (mS/cm)	0.07 \pm 0.30	0.07 \pm 0.00	46	0.266

Biotic variables	Mean \pm SD		test statistic	<i>p</i> -value
	Nikachhu (U)	Nikachhu (D)		
CPUE	165.30 \pm 171.65	58.36 \pm 106.58	5	0.313
No. of fishes (<i>n</i>)	6.67 \pm 10.17	1.83 \pm 2.56	7	0.563
No. of species (<i>S</i>)*	0.83 \pm 0.75	0.67 \pm 0.82	-0.277	0.793

* = parametric *t*-test

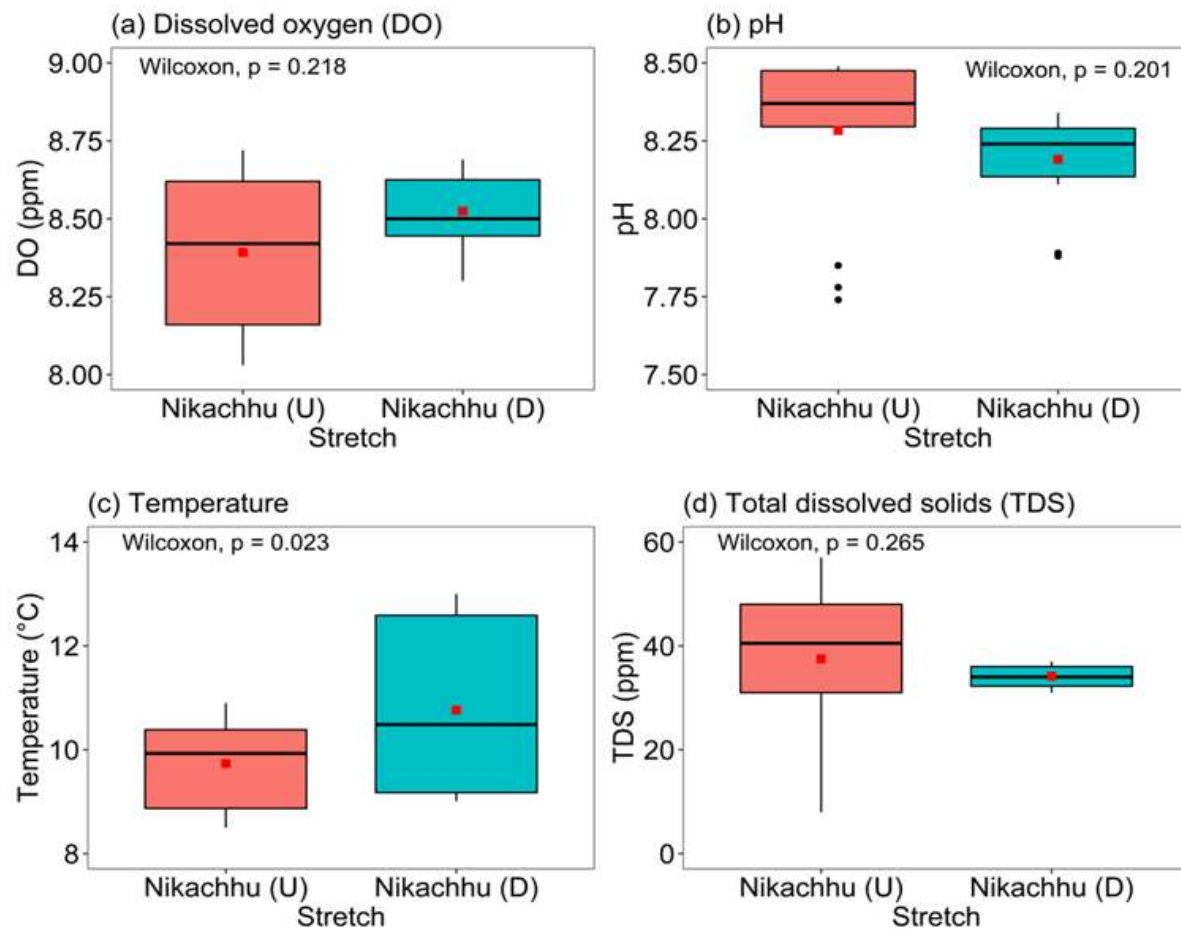


Figure 2: Comparison of basic water quality parameters between downstream [Nikachhu (D)] and upstream [Nikachhu (U)] of Nikachhu: (a) DO, (b) pH, (c) Temperature and (d) TDS. The red square indicates the group mean.

organisms, though freshwaters with a pH range of 6.5 to 8.5 are generally regarded to support a healthy, diverse and productive aquatic communities (NAS 1972).

The limited information on the ecological requirement of Common snowtrout suggests waters with pH of 7.4 – 8.2 and DO of 8 – 15 ppm to provide an excellent spawning condition in the natural environment (Chandra et al. nd; Rai et al. 2002). This indicates the suitability of most downstream sites (Supplementary graph: S1a, S1b; (> 65%, except Site 6 and 9;)) for natural spawning of Snowtrout. TDS in water indicates the concentration of dissolved ions and influences the osmotic pressure of aquatic organisms and is supposed to remain < 1000 ppm in freshwater ecosystems despite the ability of many freshwater

organisms to tolerate > 5000 ppm TDS (Boyd 2020). The observed TDS along all the sampling sites (TDS: 8 – 57 ppm; Supplementary graph: S1d) were within the permissible limit of 1000 ppm. In general, the observed pattern indicates the ability of rivers to offset changes in basic water quality parameters during the hydropower construction phase. The temperature generally increases along the river continuum and the steep elevational gradient along the Nikachhu sampling sites (≈ 1,111 m) can be attributed to the increase in temperature along the river continuum (≈ 4.30°C).

3.2 The overall pattern of fisheries diversity

This study recorded six species of fishes the entire area (≈ 42.7 km river length, Nikachhu and

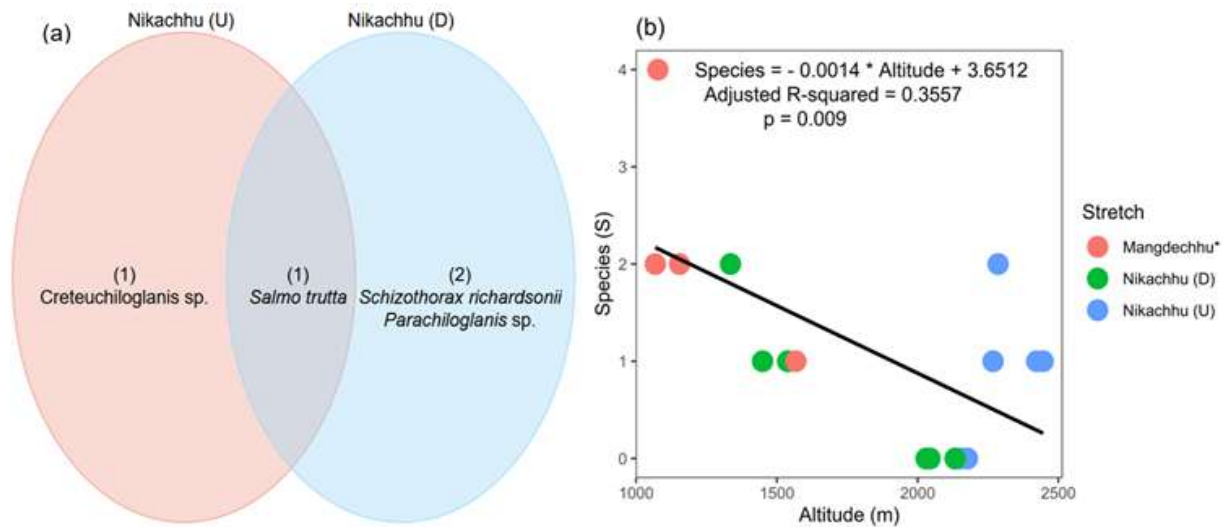


Figure 3: (a) Venn diagram showing exclusive and common species encountered from upstream [Nikachhu (U)] and downstream [Nikachhu (D)] of Nikachhu and (b) simple linear regression of species richness (S) against altitude along the entire sampling area.

Table 3: List of species recorded from Nikachhu and Mangdechhu, Trongsa during the present monitoring

Sl. No.	Species (common name)	Nikachhu	Mangdechhu*
1	<i>Salmo trutta</i> (Riverine brown trout)	✓	✗
2	<i>Creteuchiloglanis</i> sp. (torrent glyptosternid)	✓	✗
3	<i>Schizothorax richardsonii</i> (Common snowtrout)	✓	✓
4	<i>Parachiloglanis</i> sp. (torrent glyptosternid)	✓	✓
5	<i>Parachiloglanis dangmechhuensis</i> (torrent glyptosternids)	✗	✓
6	<i>Schistura</i> sp. (Stone loach)	✗	✓

Note: Nikachhu includes both upstream and downstream of Nikachhu, Mangdechhu* refers downstream of Mangdechhu Dam including its sampled tributaries, i.e. Wochenchhu.

Mangdechhu) of which four species each were recorded from Nikachhu and Mangdechhu (Table 3). The Brown trout was common species encountered both from the upstream and downstream stretches of Nikachhu, while others were exclusive, either to upstream or the downstream stretches (Figure 3a). The present study could not document earlier reported species such as *Exostoma mangdechhuensis* and *Creteuchiloglanis* sp. from downstream of Nikachhu (NRDCRLF 2020a & b). However, the presence of *Parachiloglanis* sp. were confirmed for the first time from downstream of Nikachhu. This study also records for the first time the presence of *Parachiloglanis* sp. (Mangdechhu and Wochenchhu) & *Parachiloglanis dangmechhuensis* (Wochenchhu) from the Mangdechhu stretch. On contrast earlier reported species such as *Exostoma mangdechhuensis* (Mangdechhu & Wochenchhu), *Creteuchiloglanis* sp. (Mangdechhu & Wochenchhu) & *Parachiloglanis benjii* (Wochenchhu) (NRDCRLF 2020 a & b) were not recorded from the Mangdechhu during the present assessment.

The total species recorded from Nikachhu increased over the periods of assessment. Only one species (*S. trutta*) was reported during the Environment Impact Assessment (EIA) of Nikachhu HPP (DGPC and THyE 2014). The diversity increased to three species (*S. trutta*, *S. richardsonii* and *Creteuchiloglanis* sp.) during the following assessment (NRDCRLF 2017) to four species (*S. trutta*, *S. richardsonii*, *Creteuchiloglanis* sp. and *E. mangdechhuensis*) during the monitoring conducted in 2020 (NRDCRLF 2020a & b) and then finally to five species (*S. trutta*, *S. richardsonii*, *Creteuchiloglanis* sp., *E. mangdechhuensis* and *Parachiloglanis* sp.) with the present monitoring. This increase in species richness is attributed to improvisation of sampling gear from combination of cast net, rod and line and homemade electrofisher to heavy-duty electrofisher.

The species encountered at Nikachhu post-EIA phase are either endemic (*E. mangdechhuensis*), presumed to be endemic (*Creteuchiloglanis* sp. and *Parachiloglanis* sp.) or vulnerable (*S. richardsonii*). This suggests the immediate requirement of detailed qualitative electrofishing

(in addition to an ongoing regular quantitative survey) to verify the actual species found within the current area of analysis at Nikachhu. From this experience, exhaustive qualitative electrofishing with heavy-duty electrofisher should be considered in addition to conventional cast netting to establish reliable baseline information on fisheries during the EIA of all future HPPs in Bhutan, including subsequent monitoring programs. Collaborative involvement of competent national institutions (academic or government) and implementation of standardized assessment right from the EIA will be vital in addressing the conservation concerns associated with hydropower developments.

The decrease in diversity of fishes along an elevational gradient across the the entire stretch ($R^2 = 0.399$, $p = 0.008$, Figure 3b) corroborates with findings from the Himalayas (Bhatt et al. 2012) and observations from eastern Bhutan (Changlu et al. 2021). This is likely the reason for higher H and J observed along the downstream stretch of Nikachhu (Figure 4). Although the altitudinal variation of species is significant ($p = 0.009$), narrow elevation gradient (1,077 to 2,446 m \approx 1,369 m, Table 1) and limited geographical distribution (only portion of Nikachhu and Mangdechhu, \approx 42.7 km, Figure 1) are attributed for narrow variation explained by the present regression model ($R^2 = 0.399$).

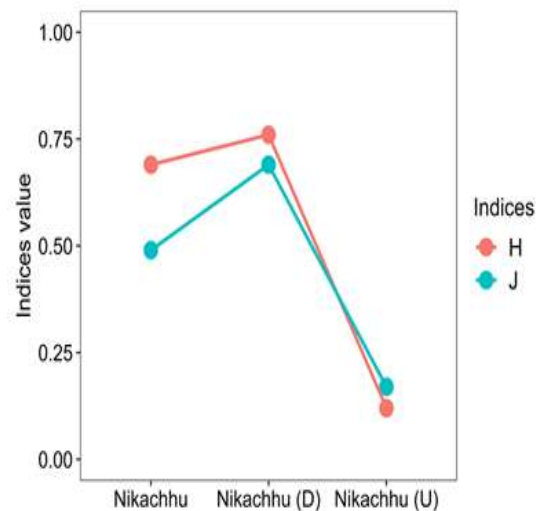


Figure 4: Comparison of Shannon diversity index (H) and evenness (J) in Nikachhu. Nikachhu is inclusive of upstream [Nikachhu (U)] and downstream [Nikachhu (D)].

3.3 Fisheries assemblage and population structure

The overall composition at the upstream stretches of Nikachhu was dominated by exotic species (Figure 5a), particularly brown trout, both by abundance (Figure 6a) and weight (Figure 6b) whereas the downstream stretches were dominated by native species (Figure 5a), mainly Snowtrout (Figure 6a, 6b). The high abundance representation of exotic fish (97.5%) reported from upstream of Nikachhu (Figure 6a) contrasts the low exotic fish composition reported elsewhere, i.e., 4% found by Santos et al. (2017) in Portugal and ~ 19% observed by Li et al. (2013) in China. However, this is attributed to pre-existing ecological disturbances, i.e., the existence of a self-sustaining population of brown trout which is now the dominant species along the upstream stretches of Nikachhu. They were introduced to rivers and lakes of Bhutan during the 1930s for the development of recreational fisheries and are well established in cold-water streams of Bhutan. Upstream dominance of insect-piscivore (Figure 5b) is subsequently attributed to the dominance of brown trout, which are insectivore and piscivore in nature.

The downstream abundance of native (Figure 5a), herbivore and insectivore (Figure 5b), and rheophilic fishes (Figure 5c) observed during this study are comparable to findings associated with the hydropower construction phase (Li et al. 2013; Santos et al. 2017). However, for the construction phase, the downstream abundance of native fishes

at Nikachhu (90.91%) is better than the native fish representation of 82% observed from impacted sites in Portugal (Santos et al. 2017). The dominance of rheophilic fishes from both the upstream and downstream stretches of Nikachhu (Figure 5c) is attributed to existence of free-flowing river, particularly at the downstream stretch as the hydropower is currently under the construction phase.

In Nikachhu, although the population structure of Brown trout (dominant upstream fish) was slightly better than Snowtrout (dominant downstream fish) (Figure 7a vs 7b), high abundance of Brown trout was observed from sites upstream of Chhuserbu (Supplementary graph: S2b, Site 1 and Site 2; i.e., 36 individuals captured above Chhuserbu vs three individuals captured between Chhuserbu and Nikachhu Dam). In recent years, the sites below Chhuserbu were subjected to yearly disturbances from landslides triggered by heavy rainfall resulting in temporary impoundment, flash floods, and mass mortalities of fishes (BBS 2020; BBS 2021). The localized destabilized areas along the river due East-West Highway expansion also made the assessment area more prone to landslides. In recent years, flash floods due to rainfall were also encountered in one of the tributaries draining within 3 km below the dam site from the right bank. The combination of these factors could have impacted the population structure of fishes downstream of Chhuserbu (i.e, Site 3 to Site 12). The inclusion of sites above Chhuserbu during the present study captured the influence of factors, other than the hydropower. This suggests the need

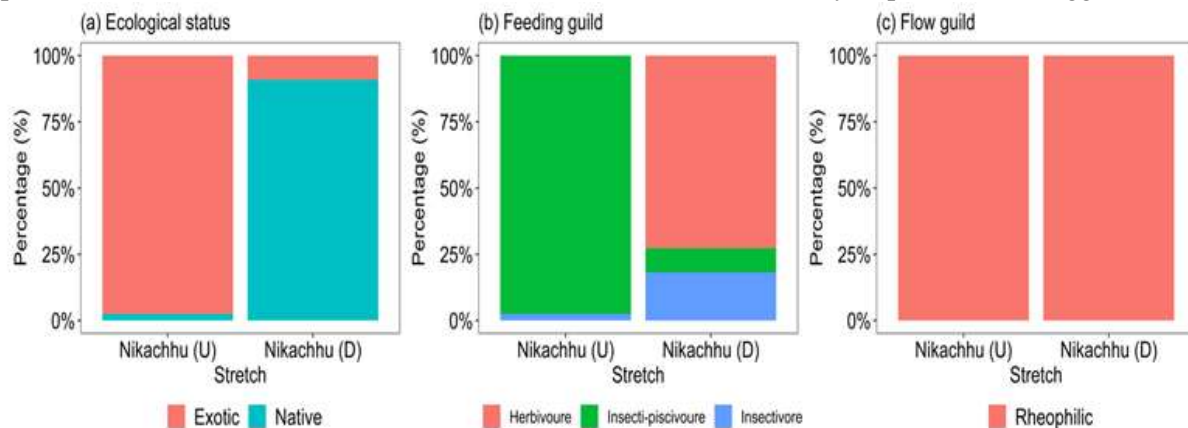


Figure 5: Structure of fish assemblage (abundance composition) in upstream [Nikachhu (U)] and downstream [Nikachhu (D)] of Nikachhu.

for adoption of an adaptive approach for any upcoming monitoring programs, especially during the construction phase and if possible, beyond.

3.4 Catch per unit effort, abundance and species richness

Although the differences were insignificant, higher values of catch per unit effort (CPUE), total abundance (number of fishes, n) and species richness (S) were observed along the upstream stretches (Figure 8a, 8b, and 8c). However, higher upstream n and subsequently the higher CPUE attributed to high catches from upstream of Chhuserbu, i.e., sites away from natural and anthropogenic disturbances (Supplementary graph: S2a, S2b; Site 1 and Site 2), indicates the influence

of natural extremities and associated impacts on the downstream composition of fisheries. In addition, the decreasing pattern of CPUE, n and S towards the dam construction site in the upstream stretch and increasing pattern of CPUE, n and S away from the dam construction site in the downstream stretch (Supplementary graph: S2a, S2b, S2c) indicates impacts of hydropower construction on the fisheries in Nikachhu. However, the comparison of spatio-temporal changes in fisheries composition since the inception of the project (2016) was difficult as the available data (presence-absence data, length information) are unreliable for further analysis considering the inconsistencies in sampling (mixture of sampling gears and lack of information on effort). One should also note less sampling

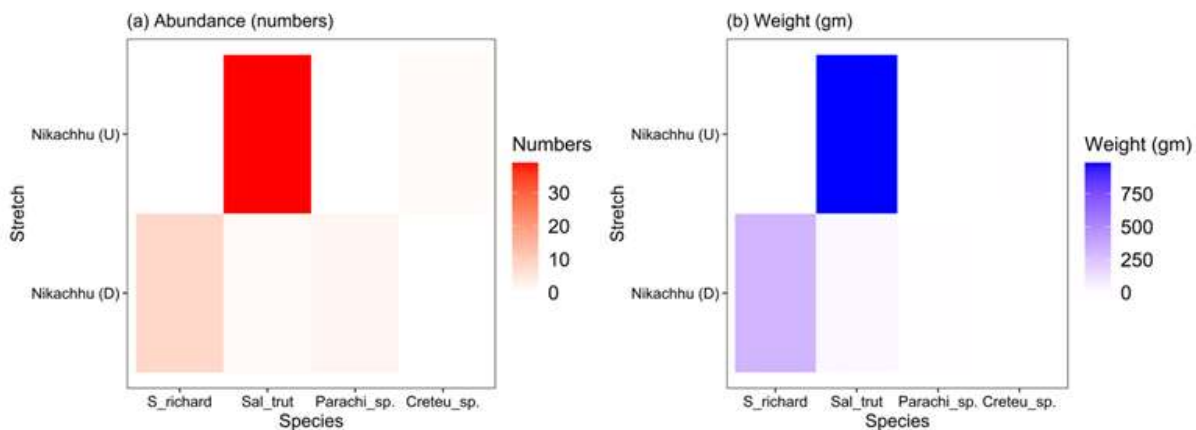


Figure 6: Heatmap showing species composition by (a) numbers (abundance) and (b) weight (gm) in upstream [Nikachhu (U)] and downstream [Nikachhu (D)] of Nikachhu. S_richard = *S. richardsonii*, Sal_trut = *S. trutta*, Parachi_sp. = *Parachiloganis* sp. and Creteu_sp. = *Creteuchiloganis* sp.

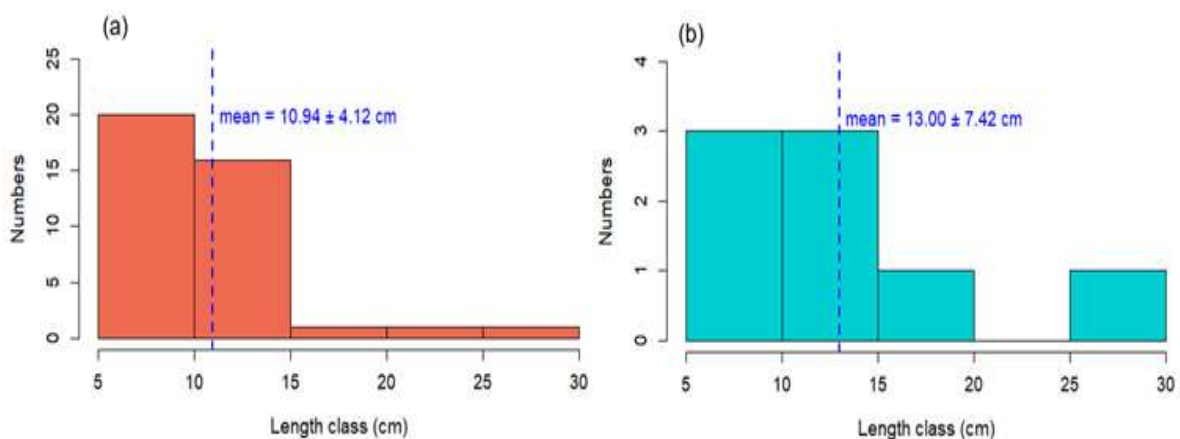


Figure 7: Population structure of dominant fish in (a) upstream (*S. trutta*) and (b) downstream (*S. richardsonii*) of Nikachhu. The dashed blue line indicates the mean length of the sample.

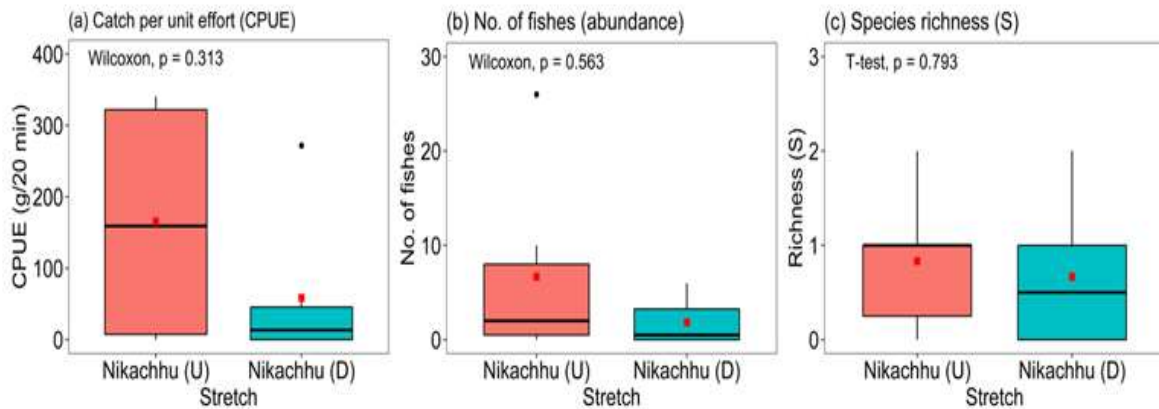


Figure 8: Comparison of biotic variables between downstream [Nikachhu (D)] and upstream [Nikachhu (U)] of Nikachhu: (a) CPUE, (b) No. of fishes (abundance), (c) No. of species (S). The red square indicates the group mean.

effort adopted during the present study. Hence, by considering the long-term consequence of hydropower development on fishes (Li et al. 2013; Santos et al. 2017) and lessons learned, future monitoring programs with standardized approaches and increased sampling efforts are crucial to realize effective conservation and management goals.

3.5 Conservation of fisheries

The occurrences of exclusive taxa at the upstream (*Creteuchiloglanis* sp.) and downstream (*S. richardsonii* and *Parachiloglanis* sp.) stretches of Nikachhu (Figure 3a) reflect Nikachhu as an important habitat for fishes. Furthermore, Snow trout, the dominant fish species downstream of Nikachhu (Figure 5a, 5b) demands conservation consideration as they are categorized as vulnerable and are threatened (IUCN 2022). Considering the rich diversity of glyptosternids endemic to Bhutan (Thoni and Gurung 2014; Thoni and Gurung 2018) and their occurrences at Nikachhu, conservation priority should cover *Creteuchiloglanis* sp. and *Parachiloglanis* sp. Furthermore, the need for critical habitat assessment (CHA) of fisheries along the Nikachhu HPP area triggered by the presence of these glyptosternids suggests, an immediate need of incorporating CHA of fisheries as a prerequisite component of EIA for any future hydropower development programs in Bhutan.

The threats to native and endemic species are expected to increase with the progression of hydropower development and intensify as other human-induced changes accelerate within the area. The Brown trout is among the 100 worst invasive species (GISD 2022) and is expected to pose threats to life strategies (Johal et al. 2021; Sharma et al. 2021) and outcompete both the migratory (Gupta and Everard 2019) and non-migratory native species through predation and competition for food (McDowell 2006). More pronounced impacts are predicated along the downstream end after the commissioning of Nikachhu HPP. However, the availability of less suitable Brown trout habitats at downstream of Nikachhu (due to steep altitudinal drop, ≈ 814 m) and reduction in habitat due to flow alteration after the dam operation may act as a barrier restricting their establishment (Almodóvar and Nicola 1999). To minimize the implications of flow reductions on native species, adequate environmental flow (e-flow) should be maintained as required by the Water Regulation of Bhutan (2014).

To offset the Brown trout population under e-flow conditions, river ranching of Brown trout should be avoided in Nikachhu, and if considered, careful attention should be given (e.g., stocking of the sterile population). Adequate attention should be given to avoid the escapement of cultured species from aquaculture facilities (planned or upcoming) into the Nikachhu and Mangdechhu, more

specifically into the dewatered stretches. Although captive breeding of native fishes can be considered with the long-term goal of developing and standardizing the breeding technologies, the subsequent release of hatchery-produced stocks into the wild requires careful analysis considering ecological and genetic consequences on native fishes (Araki and Schmid 2010; Champagnon et al. 2012; Glover et al. 2017). To sum up, these conservation measures can be summarized into three broad actions: (i) protection of existing fish diversity and habitats, (ii) preventing the introduction, accidental release, and establishment of exotics, and (iii) prior consideration of stock enhancement programs.

4. CONCLUSIONS & RECOMMENDATION

The post-monsoon community structure of fishes during the construction phase of Nikachhu HPP lacked indication of disturbances in sites away from natural and anthropogenic influence. On contrary, the population structure of dominant upstream and downstream fish species, and the pattern of species richness, abundance, and catch per unit area along the impacted sites showed indications of disturbances from natural phenomena (heavy rainfall) and associated impacts coupled with anthropogenic disturbances (road construction and hydropower development). However, the lack of long-term standardized data during the construction phase makes spatio-temporal comparison unreliable. Therefore, future programs to assess the impact of hydropower on fisheries should be an adoptive-based standardized approach, and if possible, should be integrated right from the Environment Impact Assessment. Changes are gradual and hence constant monitoring programs are crucial to identify, analyze and mitigate the anticipated impacts on our aquatic ecosystems.

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REFERENCES

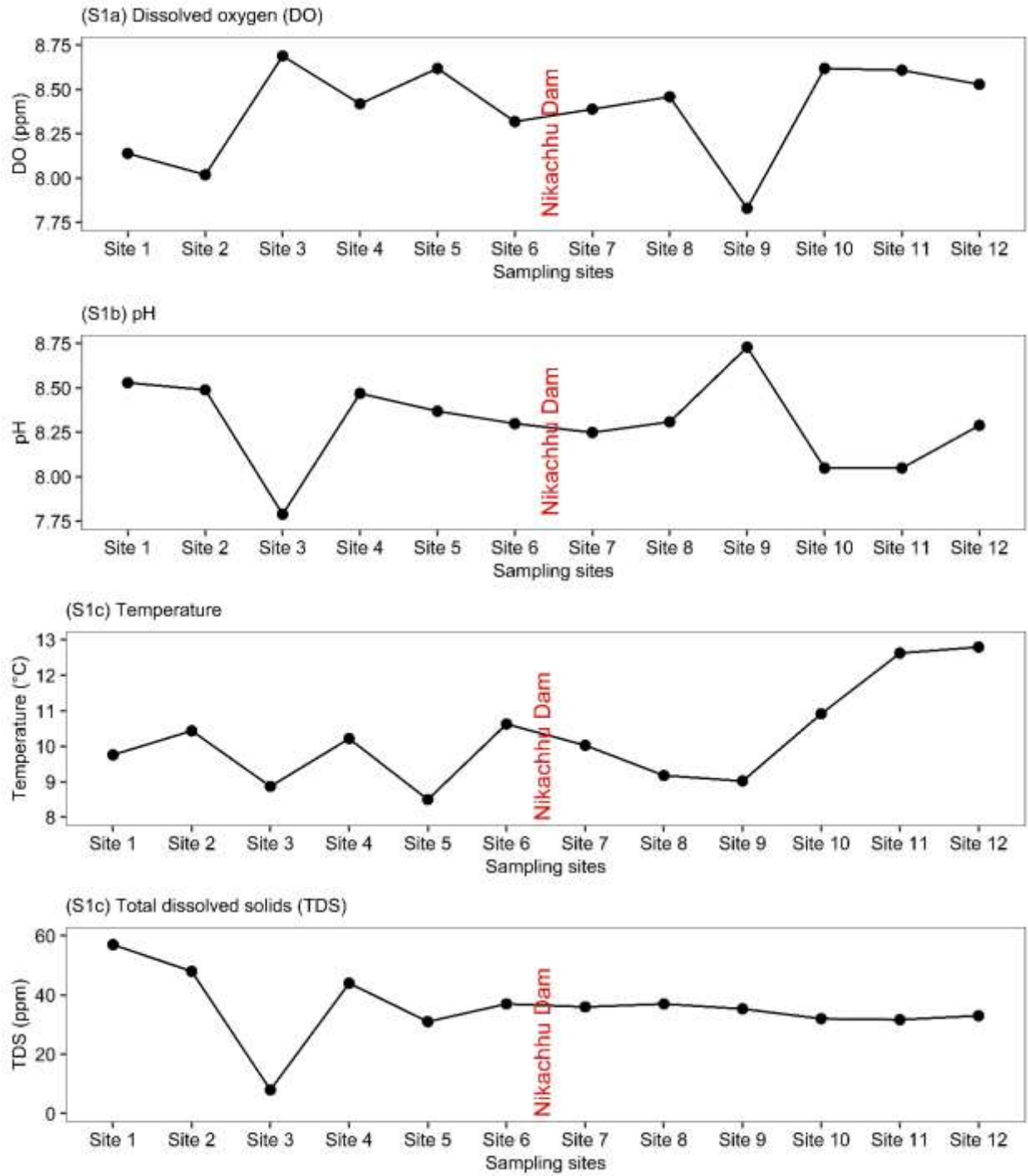
- Abujam SK, Kumar R, Darshan A and Narayan DD. (2017). Captive rearing of hill stream ornamental fishes of Arunachal Pradesh, Northeast India. *Journal of Fisheries Sciences.Com*, 11(1): 43–47.
- Alabaster JS and Lloyd R. (1981). *Water quality criteria for freshwater species*. Food and Agricultural Organization of the United Nations, Butterworth Scientific publication, London, United Kingdom.
- Almodóvar A and Nicola GG. (1999). Effects of a small hydropower station upon brown trout *Salmo trutta L.* in the River Hoz Seca (Tagus basin, Spain) one year after regulation. *Regulated Rivers: Research & Management*, 15(5): 477–484.
- Araki H and Schmid C. (2010). Is hatchery stocking a help or harm? *Aquaculture*, 308: S2–S11.
- Arantes CC, Fitzgerald DB, Hoeninghaus DJ and Winemiller KO. (2019). Impacts of hydroelectric dams on fishes and fisheries in tropical rivers through the lens of functional traits. *Current Opinion in Environmental Sustainability*, 37: 28–40.
- BBS. (2021) Roadblock information. Bhutan Broadcasting Services (BBS). <https://www.facebook.com/watch/?v=489318205699738&ref=sharing>. Accessed 25 January 2022.
- BBS. (2020) Roadblock information. Bhutan Broadcasting Services (BBS). https://www.facebook.com/permalink.php?id=133972516617260&story_fbid=3604104949603982. Accessed 25 January 2022.
- Boyd CE. (2020). Dissolved Solids. In *Water quality: An introduction*, Boyd CE, 83-118. Springer, Switzerland.
- Bhatt JP, Manish K and Pandit MK. (2012). Elevational gradients in fish diversity in the

- Himalaya: Water discharge is the key driver of distribution patterns. *PLoS ONE*, 7(9): e46237.
- CPCB. (2008). Guidelines for water quality monitoring. Central Pollution Control Board, Ministry of Environment and Forests, New Delhi.
- Champagnon J, Elmberg J, Guillemain M, Gauthier-Clerc M and Lebreton JD. (2012). Conspecifics can be aliens too: A review of effects of restocking practices in vertebrates. *Journal for Nature Conservation*, 20(4): 231–241.
- Chandesris A, Van Looy K, Diamond JS and Souchon Y. (2019). Small dams alter thermal regimes of downstream water. *Hydrology and Earth System Sciences*, 23(11): 4509–4525.
- Chandra S, Pandey NN, and Giri AK. (nd). Snow trout (*Schizothorax richardsonii* Gray, 1832). Directorate of Coldwater Fisheries Research (DCFR).
https://dcfr.res.in/download/dcfartechnologies/Schizothorax_richardsonii.pdf. Accessed 25 January 2022.
- Changlu, Norbu S, Wangchuk K, Khanal GP, Tshering S and Tshering P. (2021). Diversity of fishes across hydrological basins and elevational gradients in eastern Bhutan: A preliminary analysis. *Bhutan Journal of Animal Science*, 5(1): 27–36.
- Couto TBA and Olden JD. (2018). Global proliferation of small hydropower plants - science and policy. *Frontiers in Ecology and the Environment*, 16(2): 91–100.
- Dickson NE, Carrivick JL and Brown LE. (2012). Flow regulation alters alpine river thermal regimes. *Journal of Hydrology*, 464 – 465: 505–516.
- Dudgeon D, Arthington AH, Gessner MO, Kawabata ZI, Knowler DJ, Lévêque C, Naiman RJ, Prieur-Richard AH, Soto D, Stiassny MLJ and Sullivan CA. (2006). Freshwater biodiversity: importance, threats, status and conservation challenges. *Biological Reviews*, 81(02): 163–82.
- GISD. (2022). 100 of the world's worst invasive alien species. Global Invasive Species Database (GISD).
http://www.iucngisd.org/gisd/100_worst.php. Accessed 25 January 2022.
- Glover KA, Solberg MF, McGinnity P, Hindar K, Verspoor E, Coulson MW, Hansen MM, Araki H, Skaala Ø and Svåsand T. (2017). Half a century of genetic interaction between farmed and wild Atlantic salmon: Status of knowledge and unanswered questions. *Fish and Fisheries*, 18(5): 890–927.
- Grill G, Lehner B, Thieme M, Geenen B, Tickner D, Antonelli F, Babu S, Borrelli P, Cheng L, Crochetiere H, Ehalt MH, Filgueiras R, Goichot M, Higgins J, Hogan Z, Lip B, McClain ME, Meng J, Mulligan M and Zarfl C. (2019). Mapping the world's free-flowing rivers. *Nature*, 569(7755): 215–221.
- Gupta N and Everard M. (2019). Non-native fishes in the Indian Himalaya: an emerging concern for freshwater scientists. *International Journal of River Basin Management*, 17(2): 271–275.
- He F, Thieme M, Zarfl C, Grill G, Lehner B, Hogan Z, Tockner K and Jähnig SC. (2021). Impacts of loss of free-flowing rivers on global freshwater megafauna. *Biological Conservation*, 263: 109335.
- IUCN. (2022). Asla, *Schizothorax richardsonii*. International Union for Conservation of Nature (IUCN) Red List.
http://www.iucngisd.org/gisd/100_worst.php. Accessed 25 January 2022.
- Humphries P, Serafini LG and King AJ. (2002). River regulation and fish larvae: variation through space and time. *Freshwater Biology*, 47(7): 1307–1331.
- Johal M S, Sharma A, Dubey VK, Johnson JA, Rawal YK and Sivakumar K. (2021). Invasive brown trout *Salmo trutta* induce differential growth strategies in the native snow trout *Schizothorax richardsonii* of Himalaya: Are natives in unaltered rivers better at picking the gauntlet of invasion? *Journal of Applied Ichthyology*, 37(5): 723–734.
- Joshi KD, Jha DN, Alam A, Srivastava SK, Kumar V and Sharma AP. (2014). Environmental flow requirements of river Sone: Impacts of low discharge on fisheries. *Current Science*, 107(3): 478–488.
- Jozi SA and Maryam M. (2014). Environmental risk assessment of dams by using multi-criteria decision-making methods: A case study of the Polrood Dam, Guilan Province, Iran. *Human and Ecological Risk Assessment: An International Journal*, 20(1): 69–85.
- Jozi SA, Shoshtary MT and Zadeh ARK. (2015). Environmental risk assessment of dams in

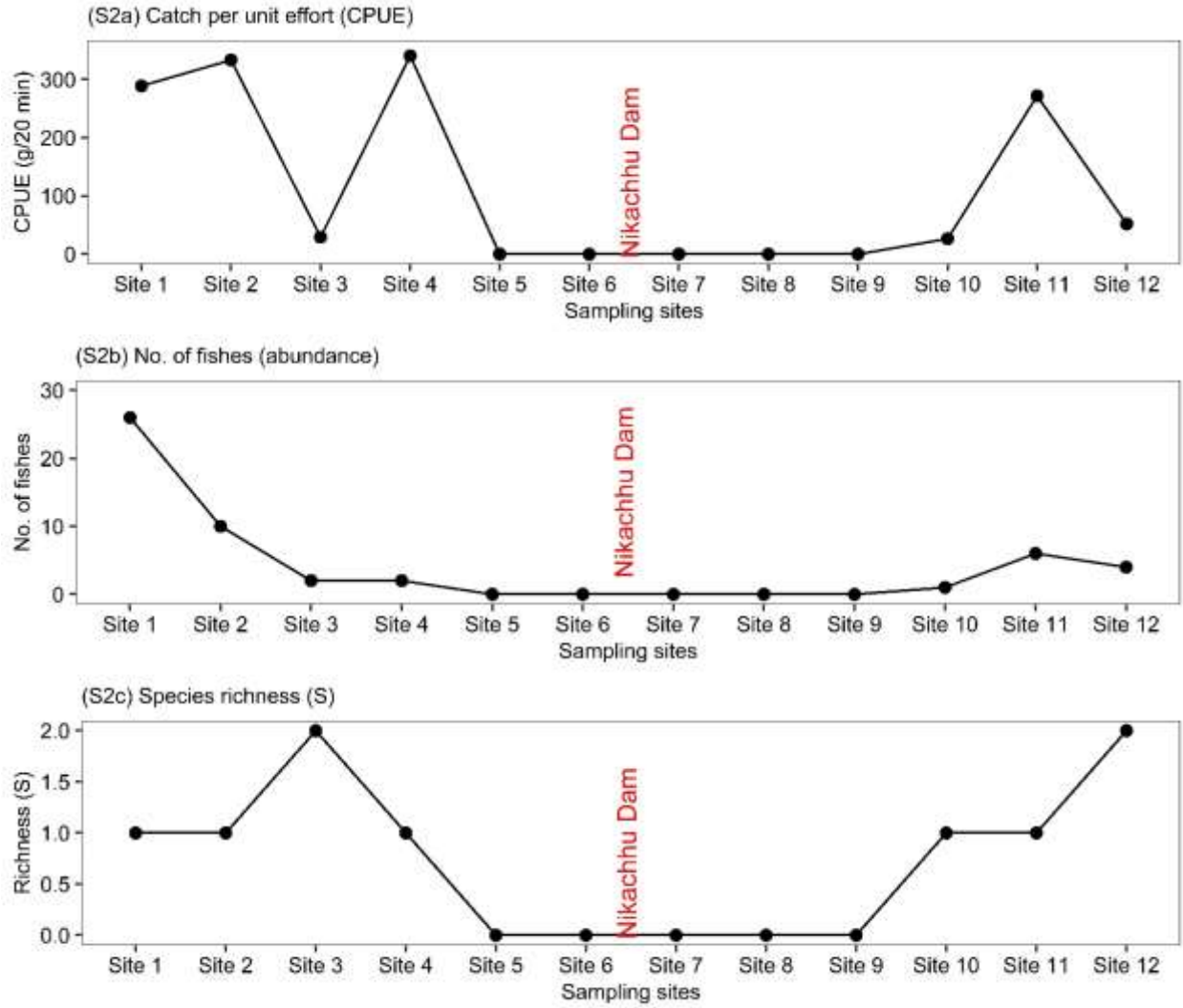
- construction phase using a multi-criteria decision-making (MCDM) method. *Human and Ecological Risk Assessment: An International Journal*, 21(1): 1–16.
- Jumani S, Rao S, Kelkar N, Machado S, Krishnaswamy J and Vaidyanathan S. (2018). Fish community responses to stream flow alterations and habitat modifications by small hydropower projects in the Western Ghats biodiversity hotspot, India. *Aquatic Conservation: Marine and Freshwater Ecosystems*, 28(4): 979–993.
- Li J, Dong S, Peng M, Yang Z, Liu S, Li X and Zhao C. (2013). Effects of damming on the biological integrity of fish assemblages in the middle Lancang-Mekong River basin. *Ecological Indicators*, 34: 94–102.
- Lima FT, Reynalte-Tataje DA, Zaniboni-Filho E. (2017). Effects of reservoirs water level variations on fish recruitment. *Neotropical Ichthyology*, 15(3): e160084.
- Meier W, Bonjour C, Wüest A and Reichert P. (2003). Modeling the effect of water diversion on the temperature of mountain streams. *Journal of Environmental Engineering*, 129(8): 755–764.
- Moreno-Arias C, López-Casas S, Rogeliz-Prada CA and Jiménez-Segura L. (2021). Protection of spawning habitat for potamodromous fish, an urgent need for the hydropower planning in the Andes. *Neotropical Ichthyology*, 19(3): e210027.
- NAS. (1972). *Water Quality Criteria. A report of the Committee on Water Quality Criteria. National Academy of Sciences and National Academy of Engineering, Washington DC.*
- NORAD. (2017). *Norwegian energy cooperation with Bhutan: a summary report. Norwegian Agency for Development Cooperation.*
- NRDCRLF. (2020a). 7th Report on fisheries development and monitoring program at Nikachhu River, Trongsa. National Research and Development Centre for Riverine and Lake Fisheries, Haa.
- NRDCRLF. (2020b). 5th Report on fisheries development and monitoring program at Nikachhu River, Trongsa. National Research and Development Centre for Riverine and Lake Fisheries, Haa.
- NRDCRLF. (2017). 4th Report on fisheries development and monitoring program at Nikachhu River, Trongsa. National Research and Development Centre for Riverine and Lake Fisheries, Haa.
- Rai AK, Pradhan BR Pradhan and Basnet SR. (2002). Present status of snow trout in Nepal. In *Cold water fisheries in the Trans-Himalayan countries*, Petr T and Sawar SB. FAO Fisheries Technical Paper 431, <http://www.fao.org/docrep/005/y3994e/y3994e0q.htm>. Accessed 25 January 2022.
- Reid AJ, Carlson AK, Creed IF, Eliason EJ, Gell PA, Johnson PTJ, Kidd KA, MacCormack TJ, Olden JD, Ormerod SJ, Smol JP, Taylor WW, Tockner K, Vermaire JC, Dudgeon D and Cooke SJ. (2019). Emerging threats and persistent conservation challenges for freshwater biodiversity. *Biological Reviews*, 94(3): 849–873.
- Rezaian S, Jozi SA and Zaredar N. (2016). Environmental risk assessment of a dam during construction phase. *Global Journal of Environmental Science and Management*, 2(4): 345–356.
- Santos J, Barbosa SC, Soares DSH, Cano-Barbacil C, Agostinho AA, Normando FT, Cabeza JR, Roland F and García-Berthou E. (2022). Assessing the short-term response of fish assemblages to damming of an Amazonian river. *Journal of Environmental Management*, 307(August 2021): 114571.
- Santos RMB, Sanches FLF, Cortes RMV, Varandas SGP, Jesus JJB and Pacheco FAL. (2017) Integrative assessment of river damming impacts on aquatic fauna in a Portuguese reservoir. *Science of the Total Environment*, 601–602: 1108–1118.
- Sharma A, Dubey VK, Johnson JA, Rawal YK and Sivakumar K. (2021). Introduced, invaded and forgotten: allopatric and sympatric native snow trout life-histories indicate brown trout invasion effects in the Himalayan hinterlands. *Biological Invasions*, 23(5): 1497–1515.
- Smith SCF, Meiners SJ, Hastings RP, Thomas T and Colombo RE. (2017). Low-head dam impacts on habitat and the functional composition of fish communities. *River Research and Applications*, 33(5): 680–689.
- Schmutz S and Moog O. (2018). *Dams: Ecological Impacts and Management. In Riverine Ecosystem Management, Schmutz S and Sendzimir J. Aquatic Ecology Series, Vol 8.*

- Springer.
- Song MY, Lee WO, Kim KH, Park YS and Bae MJ. (2016). The impact of the Goesan dam on fish communities up- and downstream. *Annales de Limnologie - International Journal of Limnology*, 52: 151–162.
- Swain S, Sawant PB, Chadha NK, Chhandaprajnadarsini EM and Katare M. (2020). Significance of water pH and hardness on fish biological processes: A review. *International Journal of Chemical Studies*, 8(4): 830–837.
- Tanir ÖZ and Fakioglu Ö. (2017). Density, Biomass and length-weight relationship of Brown trout (*Salmo trutta* Linnaeus, 1758) population in the Çoruh River Basin, North-Eastern Anatolia, Turkey. *Journal of Limnology and Freshwater Fisheries Research*, 3(3): 129–136.
- Thoni RJ and Gurung DB. (2014). *Parachilognis bhutanensis*, a new species of torrent catfish (Siluriformes:Sisoridae) from Bhutan. *Zootaxa*, 3869(3): 306–312.
- Thoni RJ and Gurung DB. (2018). Morphological and molecular study of the torrent catfishes (Sisoridae: Glyptosterninae) of Bhutan including the description of five new species. *Zootaxa*, 4476(1): 040–068.
- DGPC and THyE. (2014). Environment and Social Impact Assessment of Nikachhu Hydropower Project. Druk Green Power Cooperation (DGPC), Thimphu and Tangsibji Hydroenergy Limited (THyE), Trongsa.
- Tyagi LK, Gupta BK, Pandey A, Bisht AS, Lal KK, Punia P, Singh RK, Mohindra V and Jena JK. (2014). Length-weight relationships and condition factor of snow trout, *Schizothorax richardsonii* (Gray, 1832) from different rivers of the Himalayan region in India. *Proceedings of the National Academy of Sciences India Section B - Biological Sciences*, 84(2): 299–304.
- USEPA. (2017a). Dissolved oxygen. United States Environment Protection Agency (USEPA), Causal Analysis/Diagnosis Decision Information System (CADDIS): CADDIS Volume 2. <https://www.epa.gov/caddis-vol2/dissolved-oxygen>. Accessed January 25 2022.
- USEPA. (2017b). pH. United States Environment Protection Agency (USEPA), Causal Analysis/Diagnosis Decision Information System (CADDIS): CADDIS Volume 2. <https://www.epa.gov/caddis-vol2/caddis-volume-2-sources-stressors-responses-ph>. Accessed January 25 2022.
- Yoshida Y, Lee HS, Trung BH, Tran HD, Lall MK, Kakar K and Xuan TD. (2020). Impacts of mainstream hydropower dams on fisheries and agriculture in Lower Mekong Basin. *Sustainability*, 12(6): 2408.
- Zarfl C, Lumsdon AE, Berlekamp J, Tydecks L and Tockner K. (2015). A global boom in hydropower dam construction. *Aquatic Sciences*, 77(1): 161–170.
- Zhang P, Yang Z, Cai L, Qiao Y, Chen X and Chang J. (2018). Effects of upstream and downstream dam operation on the spawning habitat suitability of *Coreius guichenoti* in the middle reach of the Jinsha River. *Ecological Engineering*, 120: 198–208.
- Zhou W, Li X and Thomson AW. (2011). A new genus of glyptosternine catfish (Siluriformes: Sisoridae) with descriptions of two new species from Yunnan, China. *Copeia*, 2011(2): 226–241.

Supplementary graphs for site-wise variation in biotic and abiotic variables.



Supplementary graph S1. The site-wise variation in basic water quality parameter in Nikachhu (flow direction = site 1 to site 12).



Supplementary graph S2. The site-wise variation in biotic variables in Nikachhu (flow direction = site 1 to site 12).