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Research Article

Evaluation of benthic macroinvertebrate communities in upstream and downstream of Kulekhani Multipurpose Reservoir, Makawanpur, Nepal

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Abstract

Reservoir construction in the natural waterways can disrupt the structure and function of riparian ecosystems with the alteration in abundance and composition of aquatic organisms. This study assessed the variation in Benthic Macroinvertebrates (BMIs) communities in upstream (Chitlang and Seti Streams) and downstream (spillway) of Kulekhani multipurpose reservoir, Makawanpur, Nepal. Multi-habitat qualitative samplings with Ganga River System Biotic Score (GRSBIOS) index was used for the biological water quality assessment. Out of total 25 families and 8 orders of identified BMIs, Diptera and Coleoptera order are abundant whereas Oligochaeta and Odonata order are lowest. Taxa richness and abundance of BMIs were estimated to be higher upstream. Number of EPT taxa (6 to 2) and percentage of EPT (35.07 to 28.04 %) abundance were recorded in decreasing order of response toward downstream. The upstream showed a high Shannon and Simpson's diversity index. Chitlang Stream is found slightly polluted as Average Score Per Taxa (ASPT) score is calculated as 6.1 (River Water Quality Status (RWQS)-I)). Similarly, immediate downstream is critically polluted with RWQS-III whereas Seti Stream and spillway after 4 km were found to be moderately polluted as RWQS-II. Canonical Correspondence Analysis (CCA) showed that pH, temperature, and dissolved oxygen have a high influence in BMIs assemblages. The study depicts the ecological health of the stream immediately downstream of the reservoir is disturbed with presence of tolerant BMIs assemblages. The implication of the study can be in the assessment of impact caused by the reservoir in the ecological status of the water transfer.

Keywords: Benthic macroinvertebrates, dam, diversity index

Introduction

Hydropower reservoirs have many advantages as robust technology and are considered one of the most environmentally benign energy technologies (Paish, 2002). However, damming of rivers disrupts the hydrological cycle and negatively impacts the structure and function of riparian ecosystems (Rosenberg et al., 2000), as damming and impoundments alter numerous physical and chemical factors such as pH, dissolve oxygen, and water temperature (Cummins, 1979). This in turn alters the abundance and composition of biological conditions in aquatic environments (Strange et al., 1999). Dams are also the structure for habitat changes of aquatic macroinvertebrates (Armitage, 1979) and are one of the crucial biotic components in freshwater systems. The effects of dams on benthic macroinvertebrate (BMIs) communities are important because of the role that BMIs play in stream ecosystem function (Cummins & Klug, 1979).

Surface water status, concerning to Directive 2000/60/EC of the European Parliament and the Council, the status of a body of surface water determined by declined chemical and ecological status (Lanz & Scheuer, 2001) and includes consideration of biological (aquatic species including BMIs), hydro morphological (water path characteristics with dynamics), chemical and physiochemical elements (Stalzer & Bloch, 2000). A healthy river retains its ecosystem integrity which depends on its ability to maintain its structure and function, to recover after disturbance, and to support local biota in contrast to an unhealthy river which loses its capacity to provide valuable goods and services (Khanal, 2001).

Bioassessment is the study of biological indicators with physical and chemical parameters to depict the water quality and can be done by the monitoring of ecological as well as chemical components (Shah & Shah, 2012). Biomonitoring of an ecosystem is a method of observing the impact of external factors on ecosystems (Armitage et al., 1983) which is defined as "the systematic use of living organisms or their responses to determine the condition or changes of the environment" (Johnson et al., 1993). Biological monitoring is one of the best and most integrated approaches which helps to assess the ecosystems and overall environmental quality (Houston et al., 2002) which often includes the application of biotic indices and scores. Several biotic indices have been developed and about 60% of such biotic indices are based on BMIs analysis (Aanes & Baekken, 1995). The biotic index and family biotic index measure for indicating the quality of an environment based on organism types present in it. It is usually applied to assess the quality of river water. The concept was given by Cherie Stephens to measure stream pollution and its effects on its biology (Khanal, 2001). The biotic index is mainly based on two principles: the number of taxonomic groups is reduced when pollution increases

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and sensitive species disappear when organic pollution rises (Shah et al., 2011).

Globally, various types of indicators and bioindicators have been formed and used as a river water quality assessment tool to determine river health than the conventional national standards for the assessment (Barbour et al., 1996; Agboola et al., 2019; He et al., 2020). The BMIs are considered the best biological indicators for freshwater quality as they have more resistivity to contamination with their high population structure, longevity, and comparatively affordable for assessment (Rosenberg, 1992; Ko et al., 2020). BMIs are more susceptible to anthropogenic disturbances and their responses are used as indices worldwide (Kaboré et al., 2016; Edegbene et al., 2021).

Bio-assessment of Nepalese river ecosystems into water quality programs can be better incorporated through Ganga River System Biotic Score (GRSBIOS), Bagmati River System biotic score (BRSbios), Hindu Kush Himalayan biotic score (HKHbios), and Nepalese Biotic Score Extended (NEPbios-Extended) (Sharma, 2000; Sharma & Moog, 2005; Moog et al., 2008; Ofenböck et al., 2010). Incorporation of these scores is necessary to ensure the reliability of the estimation of the health status of the studied environment (Shah & Shah, 2012). All these biotic scores are assigned numeric scores ranging from 1 to 10 where lower scores and higher scores are assigned respectively to highly pollution-tolerant taxa and highly sensitive taxa. In addition to this, the average score per taxon (ASPT) is calculated to determine the river quality class (RQC) as per the assigned biotic index value (Shah & Shah, 2013). In this study, we used GRSBIOS for assessing the ecological health of rivers because this index includes many insects as well as non-insect groups and requires species-level identification for non-insects. So, the stability of the GRSBIOS index in different geographic regions makes it a promising bio-monitoring tool in Nepal (Shah et al., 2011).

Very limited study has been performed on the river water quality through biological parameters (Shah & Shah, 2013). Some limnological studies have been conducted in the Kulekhani reservoir; studies related to macroinvertebrates and water quality assessment using BMIs indicators are scant. This study tends to assess the variation in the BMIs assemblages in the upstream and downstream sections of the Kulekhani reservoir with their ecological health status.

Materials and Methods

Study area

The research was conducted in the streams draining into Kulekhani reservoir in the Makawanpur District. Two Streams namely, Seti Stream and Chitlang Stream have been considered as upstream sampling sites whereas reservoir spillway sites are considered downstream sampling sites as shown in Figure 1. The Kulekhani watershed is comprised of 43.6% forest, 34% sloping agricultural land, 9.2% shrub, 5.7% level and valley terraces, and 7.5% other land use (Adhikari et al., 2017). According to CBS (2003), the Kulekhani watershed has a total population of 31,562, with more than 80% of them depending on agriculture for their livelihood. Among them, 239 households have been involved in fish culture in 1630 cages since 2009 (Shrestha et al., 2009).

The climate of the Kulekhani watershed varies from subtropical at low land to temperate at higher elevations (Adhikari et al., 2017). The average temperature of the study area is 15˚C to 25˚C in summer whereas 10˚C to 15˚C in the winter season. As in the other regions of the country, this area also has four distinct seasons: pre-monsoon (March to May), monsoon (June to September), post-monsoon (October to November), and winter (December to February). The average rainfall within the reservoir is ~1400 mm of rainfall in the monsoon season and ~60 mm during other dry seasons, and the level of water in the reservoir changes with season and power generation (Sthapit, 1995). Details of the geographical and other characteristics of the sampling sites of the study area are presented in Table 1.

Data collection

The sampling of BMIs was carried out and hydrological and physicochemical parameters were assessed during the winter season (2019) in upstream (Seti Stream and Chitlang Stream) and downstream whereas reservoir spillway sites are considered downstream sampling sites with the 4 km of their difference. Before the sampling of BMIs, the habitat coverage of the studied riverbed within 100 m of the river stretch was estimated. At least 5 % habitat coverage of the substrate was sampled by using the multi-habitat sampling approach given by Moog (2007). A kick net of 500µm mesh size was used for the collection of BMIs from twenty micro-habitats and a composite sample was made from each site.

The width of the river was measured by using a measuring tape. Similarly, the depth was measured by a staff gauge and the velocity was measured by a current meter (Model; Gurley Price AA) in the study areas. The physicochemical parameters - pH, water temperature, Dissolved Oxygen (DO), Total Dissolved Solids (TDS), and Electrical Conductivity (EC) were measured using a handheld multi-parameter probe through the Federation and APHA (2005).

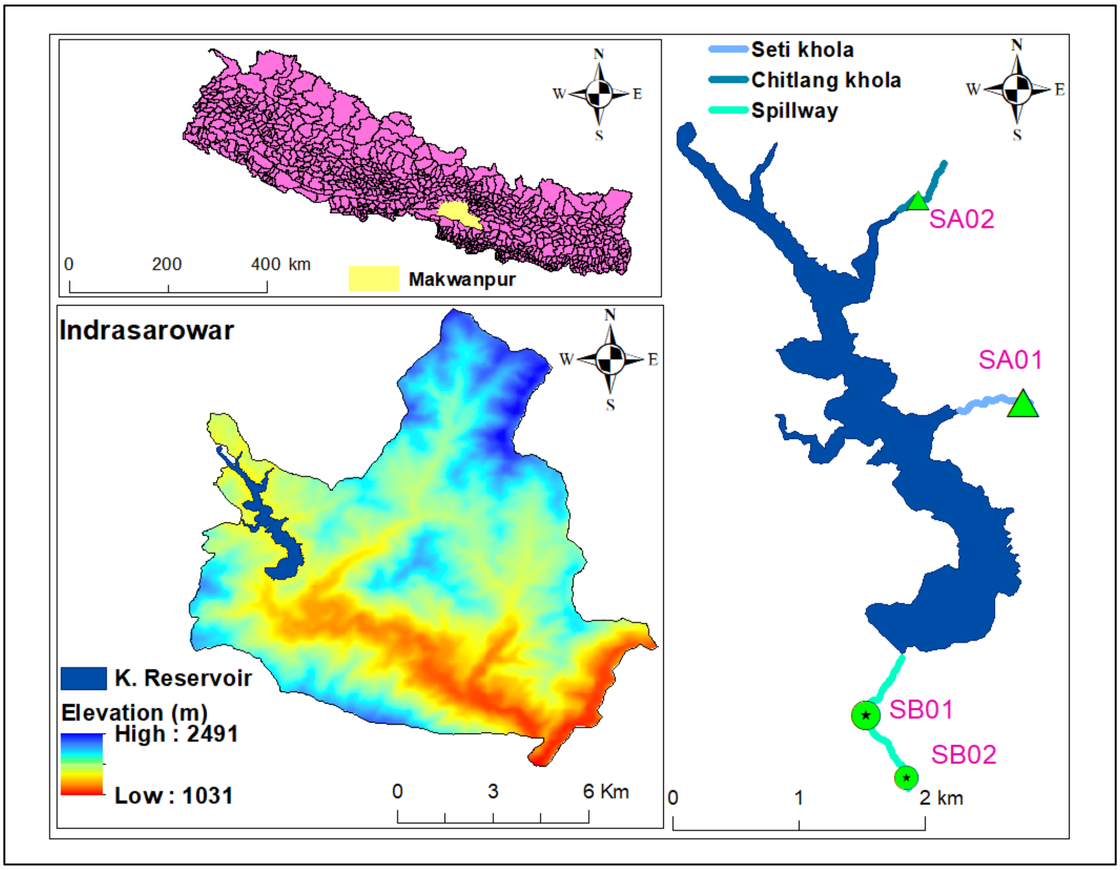

Figure 1 Map of the study area with sampling sites
(Kulekhani reservoir; Upstream - SA01 & SA02, Downstream- SB01 & SB02)

Table 1 Geographic and some features of sampling sites

Features	Upstream of the reservoir		Downstream of the reservoir	
	Seti Stream (SA01)	Chitlang Stream (SA02)	Immediate (SB01)	Spillway After 4 km of SB01 (SB02)
Location	Markhu	Markhu	Along the Kathmandu-Kulekhani Road	Along the Kathmandu-Kulekhani Road
Longitude	85°10'1.86" E	85°15'73.09" E	85°15'58.05" E	85°9'33.47" E
Latitude	27°36'28.1" N	27°62'12.14" N	27°58'38.04" N	27°34'47.81" N
Stream width (m)	1.5	5	2.5	3.5
Elevation (m)	1615	1560	1442	1295
Stream depth (m)	0.06	0.4	0.19	0.32
Velocity (m/s)	0.4	0.8	0.5	0.7
Stream bed substrate	Boulder, gravel, pebble	Boulder, gravel, pebble	Boulder, pebble, gravel, sand	Boulder, pebble, gravel, sand

Data analysis

The benthic macroinvertebrates were processed in the laboratory (Central Department of Environmental Science, Tribhuvan University) and recorded with an abundance of each sample unit in the screening protocol (Moog et al., 2008) and thus were sorted and identified at the family level using references (Dudgeon (1999); Wagner (2004); Bouchard (2004); Nesemann et al. (2006); Subramanian & Sivaramakrishnan (2007) and Nesemann et al (2011 a, b)). Then they were counted and preserved in 90% ethanol for future reference. The meaning of ecological status includes the specific aspects of the biological quality elements, for example, composition and abundance of aquatic flora or composition, abundance and age structure of aquatic fauna. Biological Water Quality Class Calculation, Ganga River System Biotic Score per Average Score Per Taxon (GRS-ASPT) (Moog et al., 2008) was calculated by dividing the number of taxa score by the total GRSBIOS score and the obtained numerical value was compared with its transformation table for evaluation of biological water quality classes of running as well as stagnant water bodies (Nesemann, 2006). The description of river water quality classes was done for GRS/ASPT value (Moog et al., 2008). Richness and abundance were evaluated by the calculation of different metrics as shown in Table 2.

Table 2 Different indices with their calculation formula

Metrics	**Calculation**	**References**
Taxa Richness	Total number of present taxa in a site	(Peet, 1974)
Total Abundance	Total number of individuals on a site	(Peet, 1974)
No. of EPT Taxa	Total sum of EPT taxa	(Peet, 1974)
Percentage of EPT abundance	(Sum of Ephemeroptera, Plecoptera and Trichoptera individuals in a site/Total abundance in a site) ∗ 100	(Peet, 1974)
<u>Diversity Indices</u>		
Shannon's Diversity Index (H)	−Σpi ∗lnpi pi = relative abundance of ith taxa	(Magurran, 1988)
Simpson's Diversity Index (D)	1−Σ (pi ∗pi) pi = relative abundance of ith taxa	(Magurran, 1988)
Evenness measures (e)	H/lnS with H = Shannon's diversity index, S = Taxa richness	(Magurran, 1988)

Multivariate analysis

The relationship between environmental variables and BMIs was evaluated through Canonical Correspondence Analysis (CCA) using the CCA function in vegan package (Oksanen et al., 2019) in the R studio version (3.1.2). The appropriate ordination technique CCA was selected and was performed amongst Hellinger transformed BMIs abundance data with the transformed explanatory variables.

Results and Discussion

Tolerance measure

Physicochemical parameters

The obtained physico-chemical parameters (Table 3) indicate the variation in the mean values among studied streams, which directly differ from the BMIs assemblages. Typically, natural pH levels fall between 6.5 and 9.0 which are varied according to the surrounding soil and vegetation (Mesner & Geiger, 2010). pH value was basic in upstream sites SA01 and SA02 (8.27 and 8.05), respectively. The downstream sections (SB01 & SB02) had lower pH values of 7.55 and 7.57, respectively.

There seem to be both direct and indirect effects in all aspects of stream ecology due to the temperature of water in it. Different organisms thrive at different water temperatures. It is regarded that the approximate upper limits range from 38°C (100°F) for fish and 50°C (122°F) for aquatic insects to 73°C (163°F) (Poole & Berman, 2001). The temperature of sampling sites above the dam sites was found to be less than below the dam sites. Increases in water temperature below dams are a known consequence of water abstraction, reducing the water's thermal capacity (Sinokrot & Gulliver, 2000). The EC was found higher (277 and 277.75 µS/cm) below dam sites and lower above the dam site (198.25 and 189.25 µS/cm). Moreover, higher EC below dams could be a consequence of higher temperatures in these sites (Gasol & Kalff, 2002). The variation in the level of conductivity may be due to the geological area through which the stream path (Dorji, 2016) and the impact of urbanization processes like sewage discharge, waste disposal or storage etc. (Glińska-Lewczuk et al., 2016; Wdowczyk & Szymańska-Pulikowska, 2020). Similarly, TDS concentration was found to be higher in below dam sites than above.

Table 3 Mean value of physico-chemical parameters

Parameters	**units**	**Mean ± SD**			
		SA01	**SA02**	**SB01**	**SB02**
pH		8.27 ±0.12	8.05 ±0.12	7.55 ±0.12	7.57 ±0.33
Temp	^{0}C	9.63 ±0.36	9.65 ±0.26	11.15 ±0.62	11.53 ±1.03
EC	µS/cm	198.25 ±1.71	189.25±3.09	277 ±4.83	277.75 ±14.84
TDS	ppm	97.5 ±1.29	97 ±1.83	138.25 ±2.5	137 ±1.83
DO	mg/L	9.27 ±0.22	9.3 ±0.29	8.09 ±0.15	6.58 ±0.33

Note; SD-Standard Deviation

The higher value of DO was recorded on site above the dam than below the dam sites. Variations in DO level might be triggered by a joint effect of temperature, photosynthesis, respiration, organic waste, aeration, and sediment concentration (Bayoh & Lindsey, 2003; Bhattarai et al., 2008). Since the distribution of many taxa is affected by concentrations of DO; it is regarded as an important component for impacting the composition of freshwater communities (Everard, 2003; Connolly et al., 2004). Generally, stream water requires 4 mg/L of DO to support diverse aquatic life (USEPA, 2008). The slightly in the DO level below the dam might be associated with anthropogenic disturbance (Akasaka et al., 2010; Dorji, 2016).

The upstream and downstream of the reservoir had evident differences in their physicochemical variables, with wide variation and extreme values in variables measured below the Kulekhani dam (Table 3). The obtained value of water temperature, TDS, and EC was found higher downstream than upstream of dam sites. Moreover, pH was found to be more alkaline upstream with a high DO value. These physicochemical variables are found directly affected the BMI's assemblages.

Tolerance measures with Ecological Health

Tolerance refers to the niche breadth or the span of the environment that an organism can cope with. Based on this it can be said that more tolerant organisms can withstand a broader range of conditions (Yuan, 2004). The GRSBIOS and ASPT value were slightly decreased below the dam as shown in Figure 2.

Table 4 represents the ecological health of different studied sites. It depicts that the site (SBO1) below the dam was critically polluted with River Water Quality Class (Sharma & Moog, 2005) (RWQC-III) i.e., critically polluted with fewer taxa tolerance score which then recovers in SB02 which was moderately polluted. The sampling sites above the dam are characterized by good ecological status specified by the high proportion of pollution-sensitive BMIs (Ephemeroptera, Plecoptera, Trichoptera, some Diptera, etc.) which have higher GRSBIOS scores. This result supported the study done by Sharma (2003) that the immediate site just below the dam got fewer scores with less water quality in the small hydropower of Tinau River.

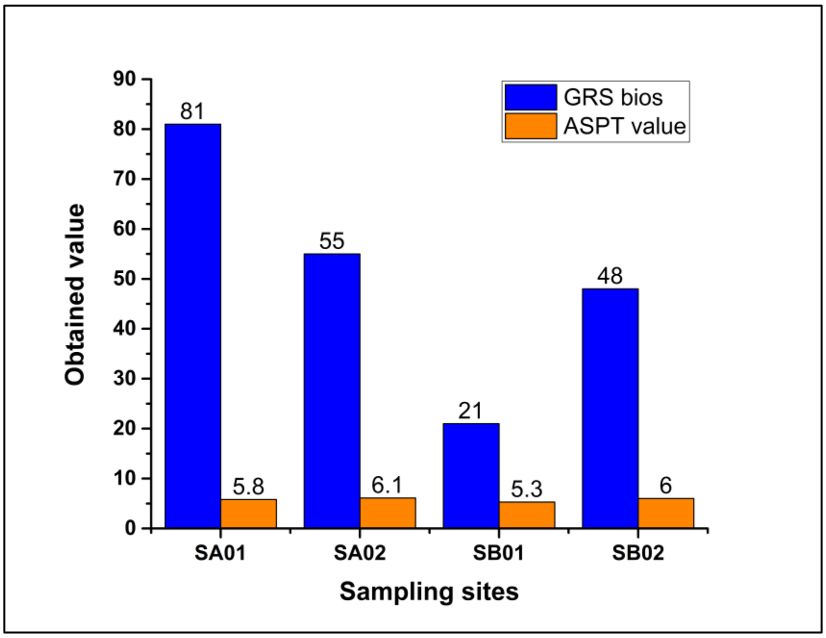

Figure 2 Tolerance measures of taxon showing with ASPT and GRS bios **values**

Table 4 Ecological health of different sites in above and below Kulekhani dam

Attributes	Above dam		Below dam	
	SA01	**SA02**	**SB01**	**SB02**
RWQC	II	I	III	II
Status	Good	High	Fair	Good
Description	Moderately polluted	Slightly polluted	Critically polluted	Moderately polluted

This study also reflects that the ecological status was degraded immediately sampling site after the dam which then recovered after four kilometers in which the river quality above the dam was found to be better than that below the dam. Previous studies have found that changes in hydrology and disruptions of the stream's natural connectivity affect the transportation of nutrients, energy, and sediments to sites below the dam, with effects on macroinvertebrate assemblages (Ward & Stanford, 1983; Petts, 1984; Petts et al., 1993; Ligon et al., 1995; Finer & Jenkins, 2012) which could be the consequences of observes low water quality below the dam sites.

Composition of BMIs

The composition measures comprise the assemblage and the relative contributions of the population of the total fauna (Houston et al., 2002). Lydy et al. (2000) have related the use of EPT taxa richness (in %) as the most illustrative tool to analyze the biological data to ensure the most effective investigation of water quality. Among the studied candidate metrics of composition measures, % of E (Ephemeroptera), % of P (Plecoptera), and % of T (Trichoptera) taxa was decreased below the dam while found higher above the dam (Figure 4). The total EPT (Ephemeroptera, Plecoptera, and Trichoptera) taxa were recorded higher above the dam sites. This result was similar to the findings of Ligon et al. (1995), Lessard & Hayes (2003), Buchberger et al. (2008), Sharma (2010), Mihalicz et al. (2019), and Ko et al. (2020), where the EPT composition on any sites was expected to decrease with the increase in disturbance because EPT taxa composition is sensitive towards temperature and flow regime (Parmesan, 2006). Hence, the families of EPT taxa were found higher above the dam.

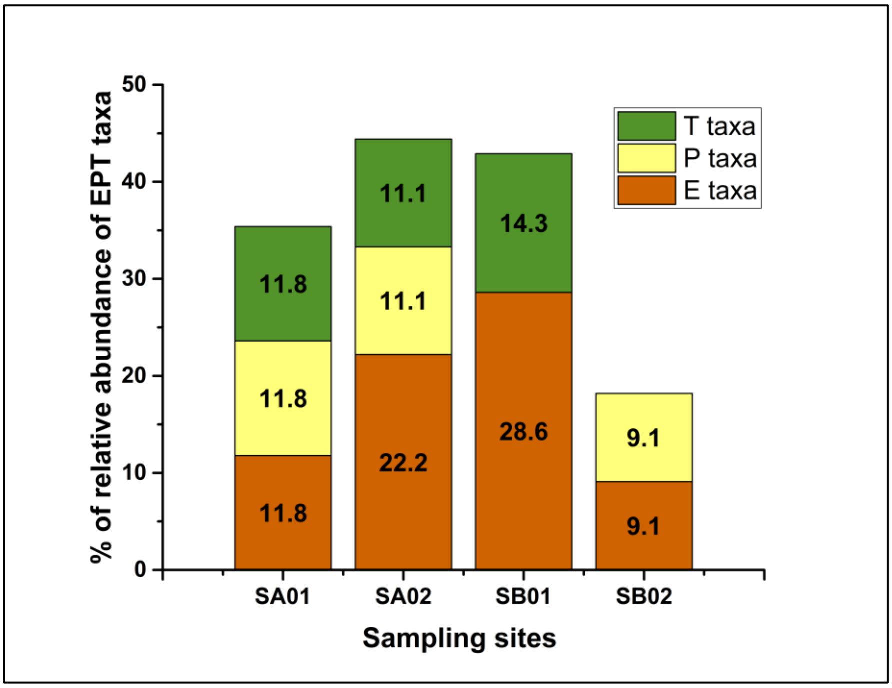

Figure 3 Percentage of relative abundance of macro-invertebrate in the study sites

Richness measures

A total of 25 Families and 8 Orders of aquatic macroinvertebrates were identified from sampling sites above and below the reservoir. As presented in Table 6, 18 Families were belonging to 8 Orders, and 13 Families belonged to 6 Orders from above and below the dam respectively. The highest number of individual macro-invertebrates was recorded from the Diptera Order and the lowest individual was recorded from the Oligochaeta order above the dam. The unique families recorded only above the dam were Ceratopogonidae, Chironomidae (not red), Nemouridae, Hydroptilidae, Heptageniidae, Coenagrionidae, and Oligochaeta. The higher individual macroinvertebrate was recorded from Coleoptera Orders and the lowest individual was recorded from Odonata Orders above the reservoir. The unique families recorded only below the dam were Chironomidae (red), Halipidae, Dytiscidae, Perlodidae, Apataniidae, Gomphidae, Hydraenidae, and Libellulidae.

The taxa richness (17 & 9), and total abundance of macroinvertebrates (211 & 98) with % of EPT abundance (35.07 & 30.61) were found higher above the reservoir (SA01 and SA02) and lower below the reservoir (SB01 & SB02) (Table 5). In the present study, the immediate sampling site below the reservoir (SB01) consisted of a lower taxa richness (6) than other sites which reflects the decrease in water quality. While the site (SA01) above the reservoir has high taxa richness but the decrease in water quality is due to the disturbed sites and lower taxa score invertebrates. Generally, the total number of taxa and biotic attributes such as total taxa richness as well as EPT taxa richness increases with the increase in water quality (Plafkin, 1989).

Table 5 Richness Measure of upstream and downstream of the reservoir

Measures	**Above dam**		**Below dam**		**Response to stress**
	SA01	**SA02**	**SB01**	**SB02**	
Taxa Richness	17	9	6	12	Decrease
Total Abundance	211	98	34	88	Decrease
No. of EPT Taxa	6	4	2	2	Decrease
% of EPT abundance	35.07	30.61	28.57	28.4	Decrease
Shannon's diversity Index	2.63	1.91	1.795	2.25	Decrease
Simpson's diversity Index	0.99	0.97	0.971	0.99	Neutral
Evenness measures	0.93	0.87	0.957	0.87	Neutral

The variation in BMIs diversity indices showed that immediate sampling (SB01) sites have high evenness measures (0.95) but less Shannon's diversity Index (1.75) than other sampling sites (Table 5). The sampling site above the dam (SA01 & SA02) showed a high concentration of the Shannon and Simpson's diversity index (2.63 & 1.91) and (0.99 & 0.97) respectively as the result showed that Shannon diversity index was higher in site SA01 which showed a high species composition, abundance and diversity were high above the dam than other sites. But the low Shannon's and Simpson's diversity index in SB01 showed less diversity and abundance which then recover in the next site (SB02). The high evenness measures obtained in the site showed that the number of species present was highly close to each other with the environment. This study is also supported by Sharma et al. (2007), where species composition and abundance of EPT are found high above the dam which depends upon heterogeneous substrate composition and higher concentration of dissolved oxygen; characteristic of less disturbed area. Similarly, the percentage of BMIs abundance was evaluated (Figure 5) were percentage of Diptera for SA01, Coleoptera for SA02, Trichoptera for SB01, and Coleoptera for SB02 was higher with 33.2 %, 45.9%, 29.4 % and 43.2%, respectively.

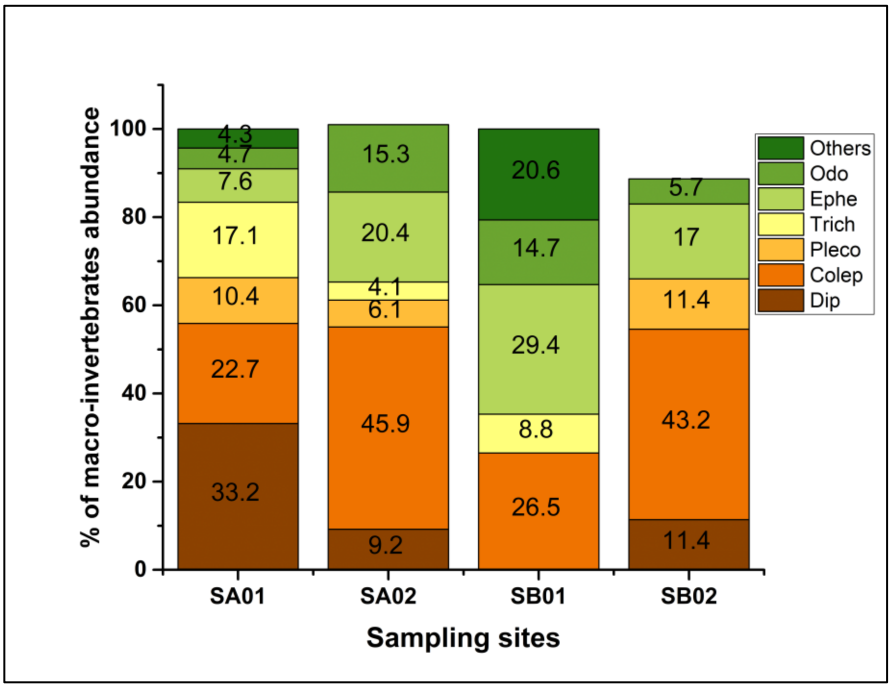

Figure 4 Percentage of BMIs abundance of sampling sites

Note: (Odo-Odonata, Ephe-Ephemeroptera, Trich-Trichoptera, Plecop-Plecoptera, Colep-Coleoptera, Dip-Diptera)

Table 6 Total number of BMIs individuals with their respective class, order, and family

Class	Order	Family	Above dam		Below dam	
			SA01	**SA02**	**SB01**	**SB02**
Insecta	Diptera	Tabanidae	16	5	-	-
Insecta	Diptera	Tipulidae	6	3	-	-
Insecta	Diptera	Chironomidae (not red)	30	-	-	-
Insecta	Diptera	Athericidae	8	-	-	10
Insecta	Diptera	Ceratopogonidae	10	-	-	-
Insecta	Diptera	Chironomidae(red)	-	-	-	10
Insecta	Coleoptera	Hydrophilidae	12	-	5	4
Insecta	Coleoptera	Elmidae	30	35	-	22
Insecta	Coleoptera	Psephenidae	6	10	-	4
Insecta	Coleoptera	Halipidae	-	-	4	2
Insecta	Coleoptera	Dytiscidae	-	-	-	4
Insecta	Coleoptera	Hydraenidae	-	-	-	2
Insecta	Plecoptera	Nemouridae	7	6	-	-
Insecta	Plecoptera	Perlodidae	15	-	-	10
Insecta	Trichoptera	Hydropsychidae	12	4	-	-
Insecta	Trichoptera	Hydroptilidae	24	-	-	-
Insecta	Trichoptera	Apataniidae		-	3	-
Insecta	Ephemeroptera	Heptageniidae	10	-	-	-
Insecta	Ephemeroptera	Baetidae	6	10	10	15
Insecta	Ephemeroptera	Ephemerellidae	-	10	-	-
Insecta	Odonata	Coenagrionidae	10	15	-	-
Insecta	Odonata	Gomphidae	-	-	5	1
Insecta	Odonata	Libellulidae	-	-	-	4
Oligochaeta	Oligochaeta	Oligochaeta	1	-	-	-
Gastopoda	-	Planorbidae	8	-	7	-
Total			**211**	**98**	**34**	**88**

Influence of environmental variables in macroinvertebrates

The macroinvertebrate acronym represents: - Ta-Tabanidae, Ti-Tipulidae, Chir (NR) -Chironomidae (not red), An-Athericidae, Cer-Ceratopogonidae, Hyd-Hydrophilidae, Eli- Elmidae, Psep-Psephenidae, Hali-Halipidae, Dyti-Dytiscidae, Hydra-Hydraenidae, Nem-Nemouridae, Perl-Perlodiadae, Hydr-Hydropsychidae, Hydrop-Hydroptilidae, Apat-Apataniidae, Hept-Heptageniidae, Bae-Baetidae, Ephe-Ephemerellidae, Coe-Coenagrionidae, Gom-Gomphidae, Libel-Libellulidae,Oligo-Oligochaeta, Plan-Planorbidae

For each of these explanatory variables (Temperature, pH and DO), a correlation was obtained with the CCA axis (Table 7). The pH has a high negative correlation with the second ordination axis and to a lesser extent temperature also displays a negative correlation with the first ordination axis. While DO has a high negative correlation with all ordination axes. For instance: BMIs families like Tabanidae, Tipulidae, and Chironomidae (Red and Not red) showed a positive correlation with pH meaning these species tend to have a larger abundance at higher pH. Similarly, Apatanidae, Gomphidae, and Halipidae have shown a positive correlation with temperature, meaning they have a higher probability of occurrence at a higher temperature. While Nemouridae (Plecoptera), Baetidae (Ephemeroptera) have shown a positive correlation with DO.

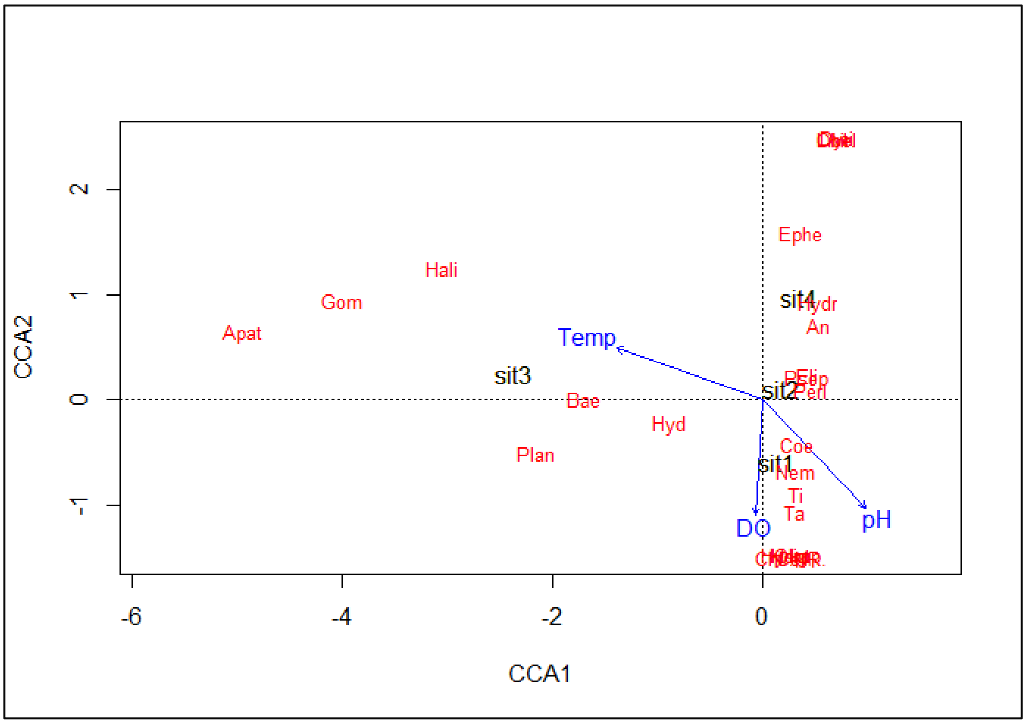

Figure 5 Canonical Correspondence Analysis (CCA) ordination plot of macro invertebrates' abundance and distribution to physico-chemical variables

Table 7 Biplot score for the constraining variables in three CCA axes

Parameter	CCA1	CCA2	CCA3
pH	0.65	-0.76	0.01
DO	-0.05	-0.81	-0.58
Temp	-0.93	0.37	0.01

The variation in macroinvertebrates assemblages was affected by physicochemical parameters supporting the studies by Armitage (1983), Lydy et al. (2000), Barbour (2001), Lessard (2003), and Dalu et al. (2017). As the study result match with other research finding where higher DO has been seen to be positively associated with EPT taxa as these taxa mostly contain sensitive organisms (Mattson, 1996). Similarly, pH and temperature have been regarded as variable responses that are seen both spatially and temporally (Petrin et al., 2007), so the study on the association of BMIs along with a pH- gradient and temperature is required to fully support the results presented here.

Conclusions

This study examined the variation of the physico-chemical parameters and BMIs in the upstream and downstream of the Kulekhani reservoir. The results showed that the water quality of upstream is good with higher taxa, abundance (total and EPT taxa), and diversity index as compared to the downstream and physico-chemical parameters as the determining factor for the BMI's assemblages. The estimated ASPT score reflect the variation in the RWQS with slightly polluted Chitlang Stream, moderately polluted Seti Stream and spillway after 4 km, and critically polluted immediate downstream of the reservoir. The CCA analyses also demonstrate the higher influence of BMI's by the physico-chemical parameters. The presence of tolerant BMIs assemblages depicts the disturbance in the immediate downstream of the reservoir. However, the in-depth analysis with the inclusion of small streams and spring which in-feed the Kulekhani reservoir and sampling from the reservoir bed are lacking and strategic basin level with the plan for maintaining the connectivity of the ecological status are necessary for the comprehensive impact analysis. This study can be the baseline for the impact assessment of the damming and impoundments in the ecological health of the waterways.

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Writing original draft: MG; Review, editing and final shape: MG and TR.

Conflicts of Interest: The authors declare no conflicts of interest.

Data Availability Statement: The data that support the finding of this study are available from the corresponding author, upon reasonable request.

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