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Dynamic Responses of Riparian Vegetation and Macroinvertebrates to Riverine Ecosystems in Dhurchhu and Choekhorchhu, Bumthang

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Abstract

A bioassessment study using macroinvertebrates and physicochemical parameters was conducted in Bumthang to compare water quality between Dhurchhu and Choekhorchhu. Systematic random sampling was used to place plots at 150 meters (m) intervals along rivers, and macroinvertebrates were collected using the kick-net method, then counted and identified. Water quality parameters were measured in the field and laboratory and compared with WHO Standards, Ambient Water Quality Criteria, and Bhutan's Drinking Water Quality Standards. A total of 2,272 macroinvertebrates from 24 families, 9 orders, and one water mite genus were recorded. *Plecoptera* (n = 1,158) was dominant, while *Anisopzygoptera* (n = 13) was least abundant. Run habitats had the highest abundance (n = 827) and cascade the lowest (n = 243). Kruskal–Wallis tests showed no significant differences among habitats in Dhurchhu ($H(3) = 1.96$, $p > 0.05$) and Choekhorchhu ($H(3) = 2.99$, $p > 0.05$). HKHbios and EPT indices indicated good water quality, associated with dominant forest cover (3,271.14 ha) within a 200 m riparian buffer. Increasing sampling points, field verification, and performing seasonal assessments are recommended to determine the effects of vegetation on water quality and macroinvertebrate diversity.

Research Article

Keywords
EPT
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LULC
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Introduction

Freshwater ecosystems, though critical for life, are among the world's most endangered habitats. According to the World-Wide Fund (WWF) (2018), 71% of Earth is covered by water, yet only 2.5% is freshwater, most of which is locked in ice or underground. These vital systems face severe threats from overexploitation, pollution, habitat destruction, exotic species invasion, and climate change. IUCN (2010) noted significant species diversity in the Eastern Himalaya's freshwater. Bhutan, despite having the highest per capita water availability, still faces water crises due to water quality degradation from anthropogenic activities (Bruno et al., 2014).

To address these threats, bioassessment provides a critical tool for the evaluation of ecosystem health. This approach uses biological indicators such as macroinvertebrates to assess water quality and environmental factors. Macroinvertebrates help to maintain urban river ecosystems' integrity and provide a basis for water quality monitoring due to their diversity, lifespan, bottom-dwelling behaviour, and sensitivity to habitat disturbances (Agouridis et al., 2015; Min & Kong, 2021; Zhang et al., 2023). Studies in Bhutan recorded 109 fish species, 18 macroinvertebrate orders, 27 phytoplankton species, and 49 zooplankton species (Gurung et al., 2017; Laini et al., 2019). Riparian vegetation enhances aquatic health but is often overlooked in studies.

Riparian vegetation plays a vital role in maintaining aquatic ecosystem health by regulating water quality, stabilising soils, and providing habitat, making riparian zones important for conservation (Agouridis et al., 2015). However, human activities and land-use changes can alter riparian vegetation, hydrology, and water quality, affecting aquatic biodiversity (Bruno et al., 2014; Kariyawasam et al., 2021). Although macroinvertebrates are widely used in bioassessment, the relationship between riparian vegetation and benthic macroinvertebrate communities remains understudied (Min & Kong, 2021; Nguyen et al., 2023; Zhang et al., 2023). Therefore, this study assesses water quality, examines macroinvertebrate diversity in relation to water quality, and evaluates the influence of riparian land-use types.

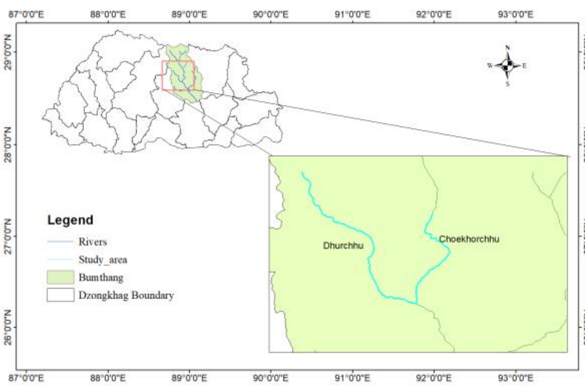
Material and Methods

Study Area

Bumthang is situated in the North-Central part of Bhutan with a total area coverage of 2,708.46 km^2. The Dzongkhag has four gewogs, Chhoekhor, Chhumey, Tang, and Ura (NSB, 2010), with altitudes ranging from 2700-4000 m (8,850-13,000 ft). Bumthang has a highland tropical climate with a dry winter climate. The average

Figure 1

Study Area Map



temperature ranges from 9.61 °C to -5.99 °C with a mean annual precipitation of about 1,488 to 2,400 millimetres annually (NCHM, 2023). Bumthang has 3,770 households and a total population of 17, 820 (NSB, 2017).

Research design

A systematic random sampling design was used, with a minimum of 20 plots per stream, resulting in a total of 40 plots across the two streams, Dhurchhu and Choekhorchhu. The distance between plots was 150 m along the stream. The sample plots represent a balance between methodological rigour and logistical feasibility for this initial study. While this number aligns with established bioassessment protocols for detecting major ecological trends, we acknowledge that it may limit statistical power for identifying subtle relationships. To mitigate this, the study employed robust non-parametric statistics and focused on intensive data collection for each plot. This approach provides a scientifically valid foundation for generating hypotheses and establishing baseline data to guide future studies in the region.

Physicochemical Parameters Sampling

Water samples were collected from Dhurchhu and Chhoekorchhu streams, analysed for water quality parameters such as hydrogen ion concentrations (pH), electrical conductivity (EC) (µS/ cm), total dissolved solids (TDS) (mg/L), and temperature (°C) measured in-situ using PCS-Testr and dissolved oxygen (DO) (mg/L) using a multi-meter probe (Asmamaw et al., 2021). Water samples were collected in HDPE (high-density polyethene) bottles pre-rinsed three times with the sample water. Bottles were labelled with date and plot ID (Alam et al., 2007), which was later transported to the laboratory for chlorine, phosphate, ammonia, magnesium hardness, calcium hardness, total hardness, and turbidity analysis (Gurung et al., 2017). Water quality was tested using different methods using the laboratory user manual prepared by Asmamaw et al. (2021) and Dorji et al. (2021).

Macroinvertebrates Sampling

Macroinvertebrates were sampled from different habitats of the river stretch using a D-frame kick net sampling approach (Asmamaw et al., 2021). In each site, sampling was done for 20 minutes within an area of 5 m from the sampling point to maintain consistency in data collection (Dunsmoor-Connor & Dunsmoor, 2017). Macroinvertebrate samples were preserved with 10% formalin and transported to the laboratory for analysis. Later, the samples were counted and identified to family level using a stereomicroscope and HKH biotic keys (Asmamaw et al., 2021). Samples were labelled with plot name, sampling date, location, and Geographical Positioning System (GPS) coordinates recorded using SW maps.

Interpolation of Water Quality Parameters

Spatial interpolation was performed using Ordinary Kriging, a method based on a rigorous statistical framework that accounts for the spatial structure and variability of the data. It can model complex spatial patterns, trends, or anisotropies by using different types of correlation or covariance functions (Krivoruchko & Gribov, 2014). Remote sensing and GIS, along with the Kriging method, were used to predict the spatial distribution of groundwater quality parameters worldwide. However, it is not popular in Bhutan. Kriging was done using ArcGIS software with the physicochemical data of water quality from study sites.

Landcover Classification

Land use land cover (LULC) classification was done using ArcGIS 10.4 software. The data were divided into two: remote sensing data and reference data. The study was based on (reference data) LULC 2016. A satellite image was obtained from the U.S. Geological Survey (USGS) database for Landsat 8, which was acquired from January 1 to December 30, 2023 (Rwanga & Ndambuki, 2017). It was selected based on availability and suitability, with cloud cover less than 30%. GPS points, which were collected from the field, were loaded in Google Earth Pro, converted to shapefiles, and used for training data during classification, which enhances the accuracy. Map accuracy was assessed using Google Earth Pro (Kamusoko, 2022).

Data Analysis

Macroinvertebrates Analysis

Diversity indices, including species diversity, species richness, abundance, and evenness, were calculated in MS Excel. Visualisation and statistical analysis were performed using SPSS, RStudio, and MS Excel. Correlation for water quality parameters and between water quality parameters and macroinvertebrate diversity was assessed using PCA in R. Kruskal-Wallis test compared habitat and macroinvertebrates abundance within the plots of two sites. Later, quality was compared using the EPT biotic index (*Ephemeroptera, Plecoptera, and Trichoptera*) (Hilsenhoff, 1988; Lenat, 1988) and the HKH (Hindu Kush-Himalayan biotic score) index (Ofenböck et al., 2010).

Formula for diversity indices:

Shannon diversity index *(H)* ………. $H' = -\Sigma (Pi * Ln\ Pi)$ ……… Eq. (1)

Species richness …………... $SR = (S-1)/ LnN$ ………………….. Eq. (2)

Species evenness ………… $EH = H'/ LnS$ …………………….. Eq. (3)

Relative abundance ………… $RA = n/N \times 100$ ………………… Eq. (4)

Where;

H' = Shannon diversity index

S = sum of genera

N = sum of all genera
n = sum of individual count
RA = relative abundance

HKHbios was calculated using the following formula:

$$\text{HKH Biotic Index} = \frac{HKHbios - Score \times Weight}{\sum Weight} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{Eq. (5)}$$

The EPT index was calculated using the following formula:

$$\text{EPT Index} = \frac{Total\ EPT\ abundance}{Total\ taxon\ abundance} \times 10 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{Eq. (6)}$$

Water Analysis

Laboratory analysis of collected water samples was done for physical and chemical parameters. American Public Health Association (APHA) standards were followed to measure physicochemical parameters of water (APHA, 2017). Turbidity (NTU) was measured following the Nephelometric method, water hardness following the EDTA method, Chloride following the Argentometric method, and Ammonia following the Phenate method (Solórzano,1969). The physicochemical parameters of water were later compared with the standards reflected in Bhutan Drinking Water Quality Standard, 2016 (NEC, 2016), Ambient Water Quality Criteria, 2020 (NEC, 2020), Drinking Water Parameters, 2014 (EPA, 2014) and WHO Drinking Water Quality Standards, following the format used by Dorji (2023).

Results and Discussion

Physicochemical variables of water quality

pH

The physiological functions of aquatic organisms are influenced by the pH of freshwater. Most surface waters have a pH range of 4 to 11, while a range of 6.4 to 8 is generally favourable for aquatic life (Seri, 2019). In this study, the pH of Choekhorchhu ranges from 8.88 to 11.81, whereas Dhurchhu ranges from 7.33 to 10.12 (Table 1). The relatively higher pH may be associated with nearby settlements, road construction, and agricultural activities (Dorji et al., 2023). In addition, increased photosynthesis can raise pH by reducing dissolved CO_2 and carbonic acid concentrations (Dodds & Whiles, 2010; US EPA, 2025).

Turbidity

The turbidity of water is defined as a measure of water clarity or cloudiness, which helps to assess environmental health and indicates the occurrence of suspended solids (WHO, 2011). Turbidity of Choekhorchhu ranges from 0.04 to

2.45 NTU, whereas that of Dhurchhu ranges from 0.04 to 2.73 NTU. The standard value of turbidity is 5 NTU. The low turbidity in the site indicates that the water is free from suspended solids (Dorji et al., 2023).

Total dissolved solids

Total dissolved solids (TDS) contribute to the measure of turbidity. WHO (2011) recommends a TDS of less than 600 mg/L for drinking water. The average TDS levels were approximately 49.01 mg/L for Choekhorchhu and 10.77 mg/L for Dhurchhu. Higher TDS can be due to anthropogenic activities and landslides in the area. It is important to monitor as it indicates eutrophic conditions (Dorji et al., 2023). H_2O Global news (2025) and U.S. EPA (2021) also acknowledge that higher turbidity in freshwater is caused by natural processes such as wind or rainfall-induced soil erosion, decomposing plant matter and algae blooms, along with human activities.

Temperature

Water temperature is a key factor influencing the distribution, abundance, and richness of aquatic organisms. The recommended optimum temperature for freshwater is below 25°C (EPA, 2021). Temperature affects dissolved oxygen, photosynthesis, and the metabolism of aquatic organisms and parasites (Dorji et al., 2023). In this study, the temperature of Choekhorchhu ranges from 3.6 to 7.5°C, while Dhurchhu ranges from 3 to 7.1°C. Such low temperatures are expected because the temperature of freshwater systems can approach 0°C during winter, particularly in colder climates (Capon et al., 2021).

Electrical conductivity (EC)

Electrical Conductivity (EC) reflects the amounts of dissolved salts in water, and higher amounts of EC will impact flora and fauna as it leads to the mobilisation of toxic chemicals. It is influenced by the presence of dissolved ions from the natural sources, such as weathering of rocks and soils or other human activities. With the increasing EC, TDS also increases. The EC of Choekhorchhu ranges from 36.8 to 77.8 µS/cm, and for Dhurchhu it ranges from 61.1 to 95.6 µS/cm.

Dissolved Oxygen

Dissolved oxygen (DO) is a key water quality parameter that influences the survival of aquatic macroinvertebrates. DO levels below 2 mg/L can reduce the fitness and survival of many aquatic organisms, and levels should generally not fall below 4 mg/L for most water uses (Dorji et al., 2023). In this study, DO ranges from 8.9 to 10.5 mg/L in Choekhorchhu and 5.87 to 8.25 mg/L in Dhurchhu. The relatively high DO levels may be attributed to seasonal effects, as DO is typically higher during winter and early spring, and to water movement, which increases oxygenation in riffles and flowing water compared to pools (EPA, 2012).

Chlorine

Chlorine is known to be toxic to aquatic organisms and impair water quality due to its ability to react with organic matter. The permissible limits of chlorine are > 0.5 mg/L for drinking waters of Bhutan (BDWQS, 2016). The increase in chlorine can be due to human-caused factors, and it increases the corrosiveness of water with sodium (Dorji et al., 2023). The chlorine level for Choekhorchhu and Dhurchhu ranges from 0.1 to 0.8 mg/L and 0.1 to 0.4 mg/L, respectively. The free residual Chlorine for drinking water standards of Bhutan ranges from 0.2 to 0.5 mg/L.

Salinity

Salinity measures the concentration of dissolved salts in water and is usually measured in ppt or percentage (%). It mostly affects the physical properties of water, such as density and conductivity. The salinity of Choekhorchhu and Dhurchhu ranges from 23.8 to 36 ppt. and 21.1 to 43.6 ppt., respectively. The salinity levels of these two sites are low and well within the range typical for freshwater, which is also indicated by the study done by Horiba (2016).

Ammonia

Ammonia (NH_3) is present in very small amounts in natural water due to microbiological activity. High concentrations of ammonia in water can be toxic to aquatic organisms, and it is a common pollutant from agricultural runoff and wastewater discharge, usually indicating the possibility of sewage pollution (Dorji et al., 2023). The ammonia concentrations of Choekhorchhu were from 0.12 to 2.45 mg/L and 0.13 to 0.19 mg/L for Dhurchhu. Mooney et al. (2019) stated that ammonia toxicity varies with temperature and pH and that it can cause chronic toxicity to tropical freshwater species at concentrations as low as 1 mg/L.

Phosphate

Monitoring phosphate levels is important as excessive amounts of phosphate in water contribute towards eutrophication, leading to algal blooms and oxygen depletion. Phosphate usually enters water from human and animal wastes (Dorji et al., 2023). The phosphate level of Choekhorchhu and Dhurchhu ranges from 0.001 to 0.024 mg/L and 0.003 to 0.05 mg/L, respectively.

Hardness (Total Hardness, Calcium Hardness, and Magnesium Hardness)

Calcium hardness measures the amount of calcium ions in water, which is considered the key component of water hardness. The magnesium hardness can impact aquatic organisms' health by preventing loss of essential ions. Total hardness is a measure of mineral content in water, which includes both calcium and magnesium hardness. It is the natural characteristics of water that help to enhance the palatability and acceptability of drinking water.

Table 1

Physicochemical Parameters with Standards and the Mean of Two Sites

Units	Standards			Sites	
	WHO	BDWQS	AWQS	Choekhor	Dhur
pH	8.2 - 8.8	6.5 - 8.5	6.5 - 8.5	9.7	9.2
Turbidity	5	5	5	1.3	0.7
Total Dissolved Solids	1500	-	25	56	44
Temperature				5.28	4.27
Electrical Conductivity	1500	-	800	0.28	0.27
Dissolved Oxygen	-	6	6	9.58	8.26
Chlorine	5	0.2 - 0.9	50	0.28	0.27
Salinity	-	-		50.4	29.5
Ammonia	1.5	-	0.05	0.16	0.16
Phosphate	1.5	-	0.5	0.008	0.031
Calcium Hardness	200	<75	200	2.04	2.22
Magnesium Hardness	150	-	200	1.83	1.82
Total Hardness	500	-	3.86	3.9	4.03

Stronger positive correlation among variables is shown by higher values close to one, while values close to negative one indicate a strong negative correlation. A value close to zero shows no association among variables. Correlation is a mutual relationship between two variables, beneficial to find a consistent association, and measures the degree of association and statistical significance of the relation between two or more water quality variables (Shukla et al., 2023).

The correlation matrix (Figure 2) shows that total hardness and magnesium hardness have a strong positive correlation (r = 0.9, $p < 0.001$), followed by TDS and EC (r = 0.7, $p < 0.001$), and TDS and Phosphate and Calcium hardness have the highest negative correlation (r = -0.4, $p = 0.01$). Saalidong et al. (2022) reported a strong positive correlation between EC and TDS, and moderate correlations between TDS, magnesium, calcium, and phosphate, which aligns with this study. However, no significant correlations were observed between pH and

Figure 2

Correlation Matrix of Water Quality Parameters of Two Sites

	Temp	EC	DO	Salinity	TDS	pH	Phosphate	Ammonia	Turbidity	Mg_H	Tot_H	Ca_H	Cl
Temp	1.00	0.24	0.46	0.27	0.13	0.37	-0.23	-0.01	0.36	-0.21	-0.22	0.01	0.12
EC		1.00	0.61	0.17	0.68	0.32	-0.16	-0.09	0.23	0.09	0.12	0.06	-0.03
DO			1.00	0.24	0.51	0.40	-0.27	-0.18	0.33	-0.09	-0.10	-0.01	-0.01
Salinity				1.00	0.12	0.13	-0.07	-0.21	0.11	-0.05	-0.05	0.00	-0.10
TDS					1.00	0.53	-0.44	0.03	0.25	0.08	0.08	-0.04	0.00
pH						1.00	-0.22	0.06	0.07	-0.01	-0.04	-0.08	0.06
Phosphate							1.00	-0.28	0.21	-0.11	-0.11	0.04	0.00
Ammonia								1.00	-0.01	-0.06	0.00	0.21	0.03
Turbidity									1.00	0.12	0.06	-0.19	0.06
Mg_H										1.00	0.95	-0.42	-0.31
Tot_H											1.00	-0.11	-0.27
Ca_H												1.00	0.19
Cl													1.00

magnesium hardness, total hardness, ammonia and total hardness, turbidity, calcium hardness and phosphate, or among DO, EC, TDS, and temperature (r = 0, $p = 1.00$).

Capturing particles, screening surface runoff, and absorbing excess nutrients before they reach streams, riparian vegetation is essential to preserving and enhancing water quality. To regulate pH, raise DO, and decrease turbidity, vegetated buffers remove nitrogen, phosphorus, and other contaminants from agricultural and populated regions (Dosskey et al., 2010; Kim et al., 2019). Additionally, riparian trees' shading regulates water temperature, promoting the best possible chemical and biological conditions for aquatic life. According to Mukherjee et al. (2024), riparian zones with high vegetation are therefore directly associated with improved water quality and general health of river ecosystems.

Macroinvertebrates Diversity

A total of 2,272 macroinvertebrates were sampled from two sites belonging to 24 families representing 9 orders and 1 water mite genus. The highest order of macroinvertebrates was Plecoptera (n = 1,158), and the least was Oligochaeta (n = 2). In terms of habitat, the highest distribution of macroinvertebrates was found in run (n = 827) and the least in the cascade (n = 243).

A total of 956 macroinvertebrate samples, including water mites, were collected, belonging to 25 families and 9 orders from 20 plots in Dhurchhu. The water mite was Sperchon (n = 7). Mean of aquatic macroinvertebrates found in this site was 47.8 (SD = 3.03). The dominant family found in this site was *Perlodidae* (n = 111, RA = 11.61%). The dominance of *Plecoptera* in this site could be because they are more diverse in the cold streams and are one of the sensitive orders of aquatic insects that occur in streams with higher DO (Bouchard, 2004; Dorji et al., 2014; Hamid, 2014).

Accordingly, from Choekhorchhu, a total of 1,316 samples of macroinvertebrates, including the water mite, belonging to 24 families and 8 orders, were recorded. The mean of aquatic macroinvertebrates found in these sites was 54.3 (SD = 4.34). The dominant order in Choekhorchhu was *Hydropsychidae* (n = 184, RA = 15.1%). The dominance could be due to their adaptivity to different ecological niches and the availability of a wide range of food sources (Fergus et al., 2023).

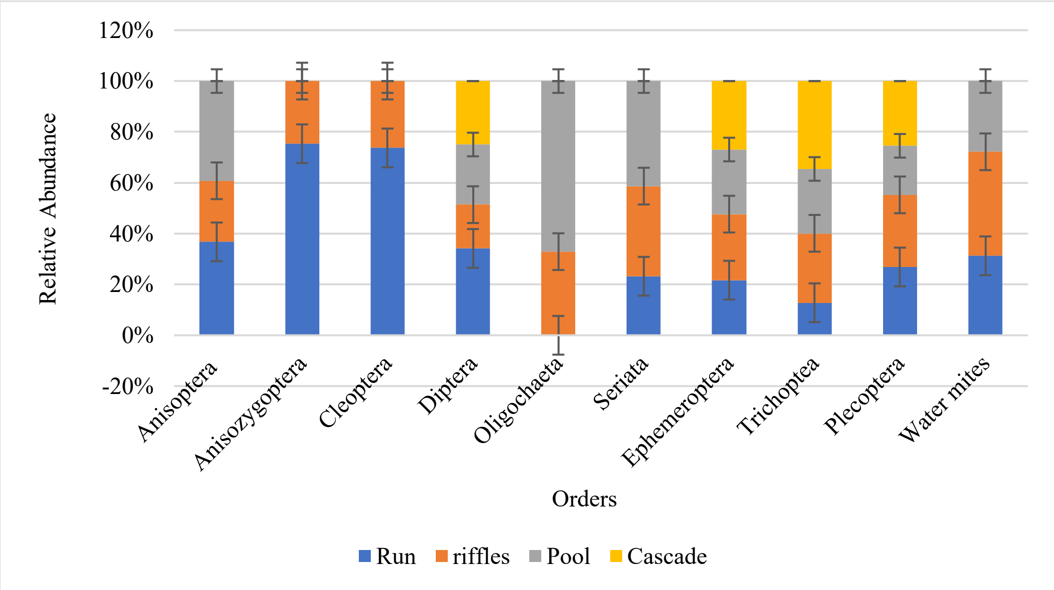
Distribution pattern of macroinvertebrates

Macroinvertebrates were sampled from four different habitats, namely, run, riffles, pool and cascade from both sites. The species diversity index using Shannon diversity shows that the diversity in Dhurchhu was H' = 1.25, and for Choekhorchhu it was H' = 1.27.

From Dhurchhu, the highest distribution was in run (n = 403) with the highest order *Diptera* (n =33) and lowest in cascade with the highest order Ephemeroptera (n = 33) (Figure 3). Wangchuk and Dorji (2018) also found that species distribution is more in run and riffles compared to the pool (Gurung et al., 2014; Wangdi et al., 2018). The abundance of *Diptera* in running water could be because they are the most diverse group found in freshwater (Rossaro et al., 2022).

Figure 3

Relative Abundance of Habitat-Wise Distribution by Order for Dhurchhu



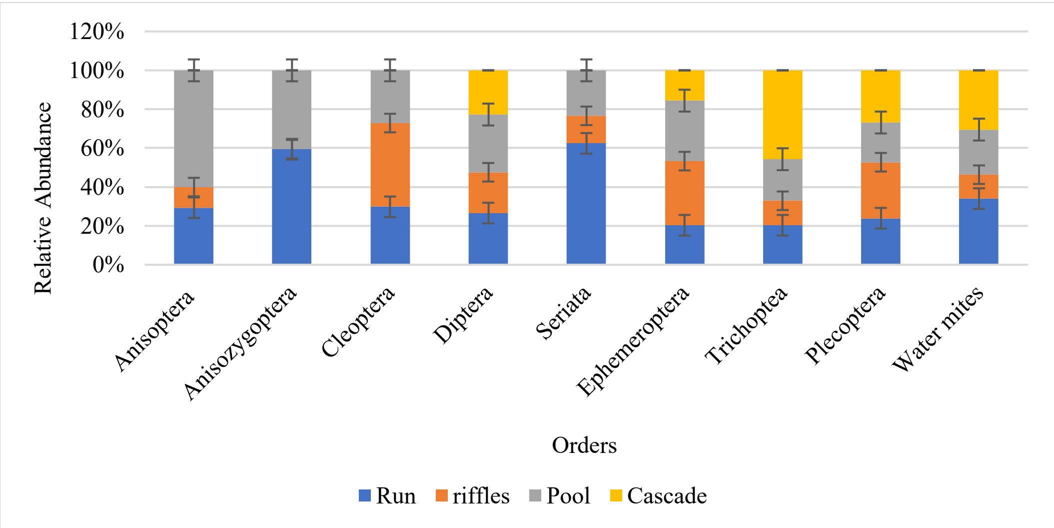
The same sample was taken from Choekhorchhu from the same habitat types. The highest was in run (n = 459) with the highest order *Plecoptera* (n = 29) and the least in cascade with the highest order *Trichoptera* (n = 34) (Figure 4). The dominance of *Plecoptera* in the stream is likely due to the low water temperature, as they are typically found in cold waters and their larvae prefer well-aerated, flowing habitats (Bouchard, 2004).

Kruskal-Wallis test compared habitat and macroinvertebrates abundance for Dhurchhu (H(3) = 1.96, p >.05) and for Choekhorchhu (*H*(3) = 2.99, p >.05). The test showed no significant relationship between macroinvertebrate abundance and habitat. As found by Khatri (2022), the non-significant result in this study could be due to a small sample size, which might have affected the statistical power, and also due to the homogeneity of habitats and temporal variability, as the study was conducted in winter months.

Riparian vegetation provides habitat structure, food sources, and stable environmental conditions. Riparian vegetation has a significant impact on aquatic wildlife. Many macroinvertebrates, which in turn support higher trophic levels like fish and amphibians, rely on the leaf litter and organic detritus from riparian plants as their main source of energy (Palt, 2022; Reich et al., 2022). The microhabitats produced by root systems and falling woody debris increase species variety, and vegetation cover controls temperature and lessens sedimentation that can suffocate benthic life. The variety and quantity of macroinvertebrates are frequently lower in degraded or sparsely vegetated riparian zones, indicating compromised ecological integrity (Rios & Bailey, 2006).

Figure 4

Relative Abundance of Habitat-Wise Distribution Pattern by Order for Choekhorchhu



Biological index of water quality

Biological water quality for both Dhurchhu and Choekhorchhu was assessed using guidelines and protocols of HKH-Bios and EPT index. The advantages of using more water quality indices are that they give an efficient general water quality of a specific area (Besacier Monbertrand et al., 2019).

Percentage (%) EPT index for Dhurchhu and Choekhorchhu

Ephemeroptera, Plecoptera, and Trichoptera are considered highly pollution-sensitive, and they can survive in well-oxygenated and clean water (Hamid & Rawi, 2017). The order Odonata is pollution-tolerant; a few are pollution sensitive; however, dragonflies and damselflies can indicate water quality as they survive in clean water. Accordingly, order *Diptera* and true bugs are relatively tolerant to pollution, and order *Oligochaeta* are pollution-insensitive, and can survive in polluted water (Zam, 2019).

The index estimates the percentage of sensitive macroinvertebrates in a sample, with the higher percentage indicating better water quality (Rai et al., 2020). EPT index percentage is higher in Choekhorchhu (74.4%), than in Dhurchhu (66.7%), showing that water quality is better at Choekhorchhu compared to Dhurchhu (Table 2), calculated using Eq. 6.

Table 2

Site-wise EPT index (%), and HKHbios index

	Sites	
Particulars	Dhurchhu	Choekhorchhu
EPT index (%)	66.7	74.4
HKHbios index	4.31	4.15

HKH biotic index and water quality

The HKHbios index assesses water quality using benthic macroinvertebrates. Originally developed by Hilsenhoff, it was adapted to the family level, with tolerance values from 0 (very intolerant) to 10 (highly tolerant) to indicate sensitivity to organic pollution (Ofenbock et al., 2010).

In this study, HKHbios values were 4.11 for Dhurchhu and 4.15 for Choekhorchhu, indicating very good water quality based on Hilsenhoff's (1988) criteria (Table 3), using eq. 5. Lower HKHbios values correspond to higher water quality, while higher values indicate poorer conditions.

Table 3

Criteria for evaluation of water quality using the family-level biotic index (Hilsenhoff, 1988)

Biotic index value	Water quality	Degree of organic pollution
0.00-3.50	Excellent	No apparent organic pollution
3.51-4.50	Very good	Possible slight organic pollution
4.51-5.50	Good	Some organic pollution
5.51-6.50	Fair	Fairly significant organic pollution
6.51-7.50	Fairly poor	Significant organic pollution
7.51-8.50	Poor	Very significant organic pollution
8.50-10.0	Very poor	Severe organic pollution

Comparing the results of the EPT biotic index and the HKH-bios index

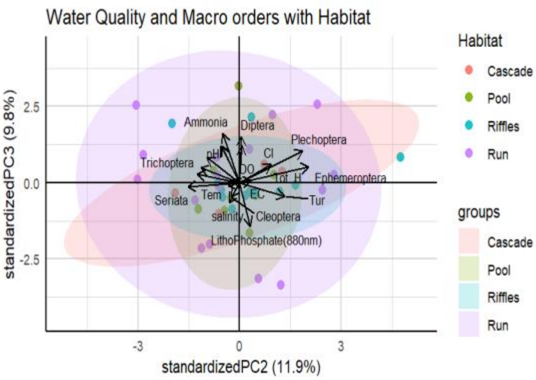
The EPT biotic index showed that Choekhorchhu has better water quality than Dhurchhu; however, the HKH biotic index showed the same water quality. The difference between the two biotic indices may be due to differences in taxonomic composition: the EPT index focuses on Ephemeroptera, *Plecoptera*, and *Trichoptera* and uses order-level metrics that may be skewed toward tolerant taxa, whereas HKH bios include a broader range of taxa (Hartmann, 2005). The HKH biotic index is affected by the family richness and evenness of the two sites.

Principal Component Analysis (PCA) between physiochemical parameters and macroinvertebrates order

PCA was used to examine correlations between the two sites, explaining 21.7% of the variance, indicating no strong overall relationship between water quality and macroinvertebrate abundance. Phosphate and turbidity were positively correlated with PC3, while Ephemeroptera, *Plecoptera*, *Oligochaeta*, total hardness, and EC were negatively correlated, suggesting that higher phosphate and turbidity reduce pollution-sensitive taxa. PC2 showed positive correlations with *Seriata*, temperature, and *Anisoptera*, and negative correlations with *Trichoptera*, *Anisozygoptera*, pH, DO, ammonia, salinity, chlorine, and water mites (Onwona et al., 2021).

Figure 5

Overall PCA for Dhurchhu and Choekhorchhu



Interpolation of water quality parameters

Interpolation was performed using altitude, longitude, and elevation data from sampling points for water and macroinvertebrate measurements, applying the Kriging method. The semi-variogram was used to visualise and analyse spatial autocorrelation in the data (Asadi et al., 2017). Using Kriging with spatial analysis tools enhances the mapping and management of water quality parameters.

Figure 6 illustrates the spatial variation of water quality parameters in the study area. Interpolated maps show higher values of DO, temperature, EC, and pH around plots 1–5 at both sites, likely influenced by human activities. EC is elevated in Choekhorchhu, possibly due to fertilisers and nearby agricultural activities, while DO is lower in parts of Dhurchhu, likely resulting from riparian forest removal by Natural Resources Development Corporation Limited (NRDCL) logging. Loss of

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riparian cover increases sunlight exposure, raising water temperature and reducing DO, as these variables are inversely related.

Figure 6

(a) Spatial Pattern of pH, (b) Spatial Pattern of Temperature, (c) Spatial Pattern of DO, (d) Spatial Pattern of EC

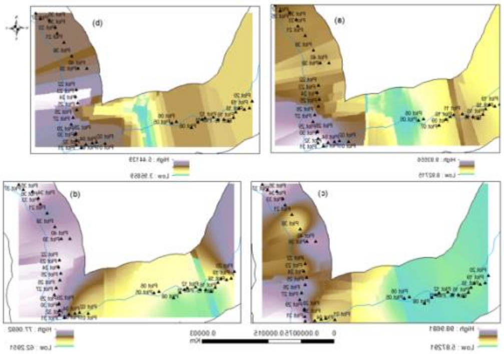


Figure 7

(a)Statistical Summary of pH, (b) Statistical Summary of Temperature, (c) Statistical Summary of DO, (d) Statistical Summary of EC

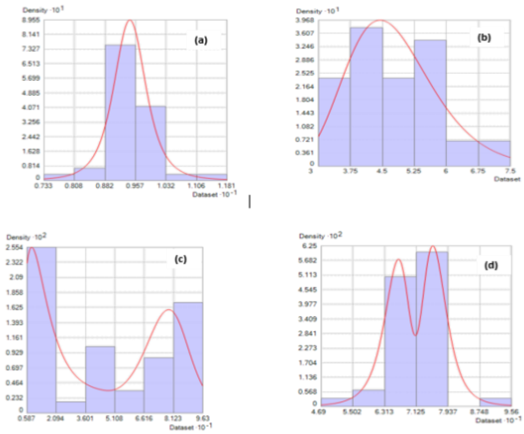


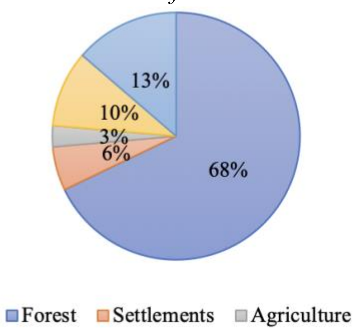
Figure 7 shows the distribution of key water quality parameters: pH, temperature, dissolved oxygen, and electrical conductivity. Himalayan rivers generally have neutral to slightly alkaline pH with near-normal distribution (Dendup & Tshering, 2022; Raxana et al., 2024). Temperature is slightly right-skewed due to altitude and canopy cover (Song et al., 2024). DO displays a bimodal pattern reflecting site differences in flow and organic matter (Phuyal et al., 2024), while EC variability is shaped by geology and land use (Dendup & Tshering, 2022). Overall, DO and EC vary by site, whereas pH and temperature are relatively stable.

Land use and land cover classification of riparian vegetation

Based on the LULC 2016, five land classes were identified according to physical observation. A total of 4,819.05 ha, which includes forest land (3271.14 ha), settlements (132.84 ha), agricultural land (132.84 ha), pasture land (276.12 ha), and barren land (658.71 ha), was classified. LULC was classified within the 200 m buffered area along the stream to determine its influence on the

Figure 8

Landcover Classification



diversity of macroinvertebrates and water quality (Verma et al., 2010). The study area has good forest cover to buffer anthropogenic factors polluting the stream.

The pie chart (Figure 8) shows the distribution of land use and land cover in the study area. Forest cover (68%) is crucial for freshwater health, as it regulates flow and filters contaminants. Barren land (13%) increases siltation and erosion, while anthropogenic land uses (19% for settlements, agriculture, and pasture) can degrade water quality through nutrient and sediment runoff, highlighting the influence of land use on freshwater ecosystems (Neary & Jackson, 2009). Accuracy assessment of the image classification yielded an overall accuracy of 98.23% and a Kappa coefficient of 98%, indicating strong agreement (Prasad, 2021).

Categories;
Kappa 1 = Perfect agreement between classification and reference data 0 < Kappa < 1 = with higher values indicating better agreement
Kappa 0 = no agreement between the classification and reference data; it's equivalent to random chance

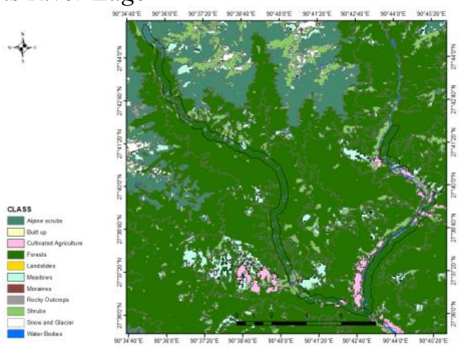
$$\text{Overall Accuracy} = \frac{\text{Total Number of correctly classified pixels (Diagonal)}}{\text{Total Number of reference pixels}} \times 100 \quad \text{. Eq. (7)}$$

$$\text{Kappa coefficient} = \frac{(\text{TS}\times\text{TCS}) - \Sigma(\text{Column Total}\times\text{Row Total})}{\text{TS}^2 - \Sigma(\text{Column Total} \times\text{Row Total})} \times 100 \ \ldots\ldots\ldots\ldots\ \text{Eq. (8)}$$

The forest cover within the 200 m river buffer (Figure 9) supports riparian services such as bank stabilisation and pollutant filtration (Dosskey et al., 2010). However, agriculture and nearby towns contribute nutrient and pollutant runoff, while adjacent grasslands and barren areas increase sedimentation and erosion, threatening aquatic ecosystems and water storage (Kim et al., 2019; Mukherjee et al., 2024). Riparian vegetation strongly influences macroinvertebrate abundance and distribution, as well as water quality. The good water quality of Dhurchhu and Choekhorchhu is likely linked to higher forest cover in the study area. Reduced forest cover and human disturbances, such as road construction and agriculture, are associated with lower macroinvertebrate diversity (Dorji et al., 2014). Conversely, areas with minimal human impact and intact riparian forests support higher

Figure 9

A Map Showing the Research Area and the Land Use Around It Within 200 m of its River Edge



macroinvertebrate diversity, which is essential for aquatic organism survival (Gurung, 2017; Mokgoebo, 2019).

According to Bhutan's Forest and Nature Conservation Rules and Regulations (FNCRR, 2023), a minimum 30 m river buffer must be maintained to protect riparian ecosystems and water quality. This buffer reduces soil erosion, safeguards aquatic habitats, and supports biodiversity, with tree cutting generally restricted to minimise ecological damage. Riparian vegetation provides food, shelter, and habitat for aquatic organisms, while its removal increases sedimentation and alters hydrological flow. Changes in land use along rivers can elevate pollution, favour pollution-tolerant taxa and reduce native species. Wangchuk et al. (2018) also reported significant differences in macroinvertebrate diversity between forested and degraded lands.

Riparian zones are essential buffers that require protection from forestry, agriculture, and water sector impacts. Land use and grazing can reduce their ability to filter pollutants and support aquatic biodiversity (Dosskey et al., 2010; Kim et al., 2019). Strengthening river resilience in Bhutan calls for adaptive buffer standards, nature-based solutions, and macroinvertebrate-based monitoring (Mukherjee et al., 2024). These buffers stabilise sediments, filter runoff, and safeguard freshwater biodiversity (Grill et al., 2019). Legally enforced zones and macroinvertebrate monitoring provide cost-effective tools for adaptive management and enhancing ecosystem resilience (Albert et al., 2021; Sweeney & Newbold, 2014).

Conclusion and Recommendation

A bioassessment of Dhurchhu and Choekhorchhu rivers was conducted to evaluate the influence of land use and land cover (LULC) on water quality and macroinvertebrate diversity. A total of 2,272 macroinvertebrates were recorded, representing 24 families across 9 orders and 1 water mite genus. Plecoptera was the most abundant order (n = 1,158) and Oligochaeta the least (n = 2). Habitat-wise, macroinvertebrates were most abundant in runs (n = 827) and least in cascades (n = 243). Water quality assessment using the HKHbios and EPT indices indicated good water quality, with HKHbios values of 4.31 (Dhurchhu) and 4.15 (Choekhorchhu) and EPT indices of 66.7% and 74.4%, respectively.

LULC analysis within 200 m buffers showed a total area of 4,819.05 ha, dominated by forest cover (3,271.14 ha) and minimal agriculture (132.84 ha). Map accuracy was high (98.23%) with a Kappa coefficient of 98%, confirming the link between forest cover and good water quality. Effective management of buffer areas should include restoring native riparian vegetation, controlling sediment and nutrient runoff, and regulating grazing to prevent bank erosion (Palt, 2022; Reich et al., 2022). Macroinvertebrate monitoring alongside Sentinel-2 imagery and community-based restoration offers an economical approach for adaptive management (Rios & Bailey, 2006).

The study's findings are based on limited sampling points; increasing sample size and conducting seasonal assessments are recommended to better understand riparian vegetation impacts on water quality and macroinvertebrate diversity. Additional statistical analyses could uncover subtle patterns between water quality, biodiversity, and land use.

By highlighting the role of legally mandated riparian buffers and their vegetation structure, the study provides evidence to guide environmental policy (Sweeney & Newbold, 2014). Managers can use macroinvertebrates as cost-effective bioindicators to identify pollution hotspots and prioritise restoration, including native replanting (Shilla & Shilla, 2012). This approach can be applied across the Himalaya to support locally calibrated river health assessments, climate-resilient conservation, and transboundary water governance (Ofenböck et al., 2010).

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