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Cyprinid (Teleostei: Cypriniformes) diversity and assemblage in south-central Bhutan

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Abstract

Cyprinidae (Teleostei: Cypriniformes) is the predominate family among Bhutan's freshwater fishes, yet significant gaps exist in the knowledge of their diversity and assemblage. Cyprinids were sampled from 54 plots across four river basins (Aiechhu, Jaldakachhu, Mangdechhu, and Punatsangchhu) using systematic sampling at an interval of 5 km. A total of 850 individuals belonging to 22 species and 13 genera were recorded. Aiechhu had the highest diversity and evenness (H'= 1.66±0.28, J'= 0.95±0.03), followed by Punatsangchhu (H'= 1.58±0.34, J'= 0.94±0.04). Meanwhile, Jaldakachhu and Mangdechhu exhibited the lowest diversity and evenness (H'= 0.90±0.66, J'= 0.84±0.54 and H'= 0.54±0.70, J'= 0.38±0.49, respectively). There was a significant difference in diversity among river basins (χ^2 (3)= 20.98, p < 0.001) with specific variation between Mangdechhu and Punatsangchhu (Z= 3.80, p= 0.00), Mangdechhu and Aiechhu (Z= 3.35, p= 0.01), and Jaldakachhu and Punatsangchhu (Z= 2.83, p= 0.03). Canonical correspondence analysis indicated that cyprinid assemblage was significantly correlated with environmental variables (r= 0.94, p= 0.001 in axis 1 and r= 0.82, p= 0.001 in axis 2) explaining 77% of variance. Axis 1 was positively correlated with temperature (r= 0.92), total dissolved solids (r= 0.53), conductivity (r= 0.51), and salinity (r= 0.39) and negatively correlated with elevation (r= −0.69). Axis 2 was negatively correlated with total hardness (r= −0.82) and dissolved oxygen (r= −0.65). Cluster analysis identified three clusters based on dominant species. Further studies exploring other river basins of Bhutan are needed to better understand the ecological dynamics of cyprinids in Bhutan.

Key words: Biodiversity index, environmental variables, Himalayas, ichthyology

Introduction

Bhutan, located in the Eastern Himalayas, has abundant water resources (Tariq et al., 2021). The country has over 2,674 glacial lakes and more than 700 major glaciers all located above 2,200 meters above sea level (m asl) (Ukita et al., 2011). These glacial lakes and glaciers are sources to 3,128 rivers and rivulets, forming five major (Amochhu, Wangchhu, Punatsangchhu, Mangdechhu, and Drangmechhu) and five minor (Jaldakachhu, Aiechhu, Nyera Amari, Jomori, and Merak-Saktengchhu) river basins (United Nations Development Programme, 2023). According to Wangmo and Rai (2019), the total length of these basins and their tributaries is estimated to be about 7,200 kilometers (km), creating diverse habitats that are an important natural resource for the region.

These aquatic ecosystems are not only vital for supporting the livelihood of local communities, but they also play a crucial role in sustaining the aquatic biodiversity of Bhutan (Dorji and Gurung, 2017). The rivers are home to a multitude of aquatic flora and fauna, including a diverse array of freshwater fishes. The basins host over 127 fish species from 8 orders (Anguilliformes, Beloniformes, Cypriniformes, Perciformes, Salmoniformes, Siluriformes, Synbranchiformes, and Tertadontiformes) and 24 families (Dorji and Tenzin, 2023; Sagar et al., 2023). The majority of the species belong to the orders Cypriniformes and Siluriformes, signifying the importance of these orders in providing sources of animal protein, game, and aquaculture (Thai et al., 2007; Nikam et al., 2014; Gurung and Thoni, 2015). The family Cyprinidae (order Cypriniformes) is distinguished as the most dominant in Bhutan, with forty species recorded nationally, including seven exotic species introduced for aquaculture (Gurung and Thoni, 2015). Notably, Cyprinidae includes globally threatened species such as the Golden Mahseer (*Tor putitora* Hamilton), Snow Trout (*Schizothorax richardsonii* Gray), and Copper Mahseer (*Neolissochelius hexagonolepi*s McClelland), highlighting its critical role in conservation efforts.

However, modern industrial development and rapid urbanization have affected cyprinid populations (Shen et al., 2016; Zeng et al., 2022). For instance, threats to *Tor putitora* have been increasing over the years from indiscriminate fishing, habitat destruction, and hindrances in migratory path due to existing and planned hydropower dams. Over time, the spawning habitats and environments for cyprinid species have dramatically changed, posing a significant threat to the survival of species within the family (Roberts and Britton, 2020). In the context of Bhutan, native cyprinids such as *Schizothorax richardsonii* and *Neolissochelius hexagonolepis* confront severe threats from the introduced carnivorous species Brown Trout (*Salmo tuttra* Linnaeus). Despite the popularity of Cyprinidae in the aquarium trade, new species in the family continue to be discovered (Tangjitjaroen et al., 2023). However, the family's extensive diversity also makes it susceptible to illegal trade practices, leading to disturbances in ecological equilibrium (Collins et al., 2012). Furthermore, additional studies are required to better understand the ecology of the family in Bhutan (Gurung and Thoni, 2015).

The knowledge of species classification, identification, and assemblage is vital in areas subject to biological and conservation efforts to prevent confusion, misinterpretations, and misunderstanding of environmental ecology (William et al., 2006; Shen et al., 2016; Keat-chuan Ng et al., 2017). Despite extensive body of research on cyprinids that explores morphological, molecular, and ecological aspects of the family (Durand et al., 2002; William et al., 2006; Yang et al., 2015; Nelson et al., 2016; Shen et al., 2016; Wang et al., 2019), significant gaps exist in the knowledge of the diversity and assemblage of cyprinids in Bhutan. Therefore, this study aims to address these gaps by assessing the diversity, distribution, abundance, and assemblage of cyprinids in Bhutan as a step towards improving taxon identification and conservation.

Material and Methods

Study area

This study was conducted in six districts (Dagana, Samtse, Sarpang, Trongsa, Tsirang, and Zhemgang) in south-central Bhutan covering two major (Mangdechhu and Punatsangchhu) and two minor (Jaldakachhu and Aiechhu) river basins (Fig. 1) chosen for their diverse ecological zones, inclusion of biological corridor, presence of hydropower projects, and status as transboundary rivers. The basins provided insights into ichthyofaunal biodiversity, environmental impacts, and conservation strategies in freshwater ecosystems of Bhutan, further highlighting their importance for research and management efforts.

The Aiechhu originates from Black Mountain located in central Bhutan (Dorji and Tenzin, 2023). The river falls within the convergences of three ecologically diverse protected areas of Bhutan which are connected by Biological Corridor 03 (BC 03) (Tenzin et al., 2021). The habitat is predominantly subtropical broadleaf forest and receives an average precipitation of 3,500–5,500 mm (Tenzin et al., 2022). It is home to 28 freshwater fishes belonging to 11 families (Tenzin, 2022).

The Jaldakachhu is a transboundary river that originates from Sikkim, flows through the Samtse district for approximately 40 km, re-enters India, and passes through Bangladesh. The basin is characterized by the prevalence of warm broadleaf forests. The elevation of the river ranges between 607–1,582 m asl (Passang, 2018).

The Mangdechhu River originates from Gangkar Puensuem, which at 7,546 m asl is Bhutan’s highest mountain, and facilitates the 720 megawatt (MW) Mangdechhu Hydropower Plant (Ugyen Wangchuk Institute for Conservation and Environmental Research [UWICER], 2017). *Daubanga grandiflora*, *Syzygium formosanum*, and *Pinus roxburghii* are some of the prominent flora identified within the Mangdechhu basin. Similarly, the Punatsangchhu basin begins from the confluence of two major rivers (Mochhu and Pochhu), originating from the Jigme Dorji National Park (JDNP) in northwestern Bhutan. It has a total stretch of approximately 320 km spanning Punakha, Wangdue Phodrang, Tsirang, and Dagana districts (Khandu et al., 2022). The basin facilitates two major hydropower projects (Punatsangchhu Hydropower Project I and II). It is dominated by warm broadleaf forest (67–500 m asl) and mixed vegetation of both chir pine and broadleaved forest (500–1,000 m asl) (Royal Society for Protection of Nature [RSPN], 2022).

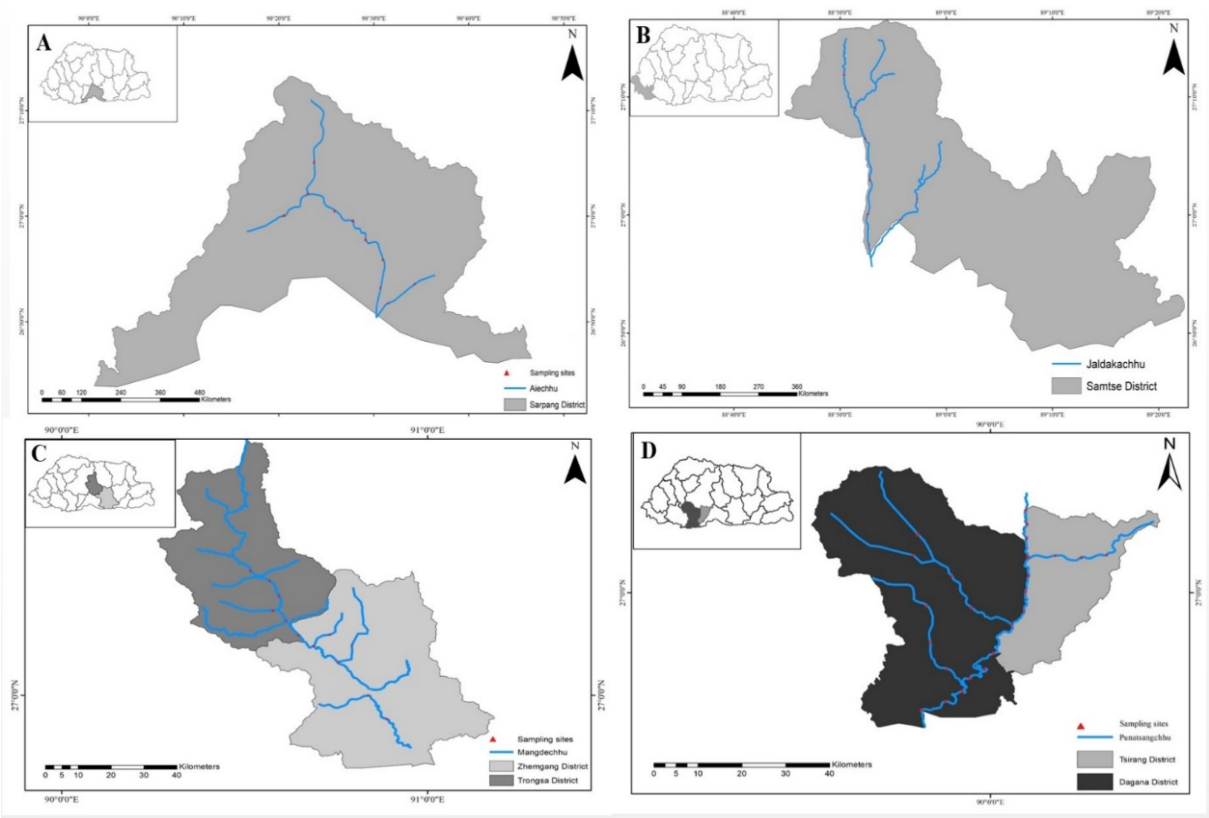

Figure 1: Study area showing the sampling sites, river basins, and districts. (A) Aiechhu basin in Sarpang district, (B) Jaldakachhu basin in Samtse district, (C) Mangdechhu basin in Trongsa and Zhemgang districts, and (D) Punatsangchhu basin in Dagana and Tsirang districts.

Sampling design and process

Hydrological maps were generated using Google Earth and river basins were stratified into sampling plots (Changlu et al., 2021). A total of 54 sampling plots were identified at five-kilometer intervals along river basins and their tributaries. Within each sampling plot, a 300 m sampling stretch was laid and sampling was conducted in multiple habitats including pools, riffles, cascades, and runs (Wangchuk et al., 2017; Wangmo and Rai, 2019; Changlu et al., 2021). Sampling was performed diurnally (09:00 to 17:00). Samples were collected using various types of fishing gear including gill net, cast net, seine net, electro shocker (Honda GCV 160cc, Tokyo, Japan), and fishing rod and line.

Environmental characterization

Elevation was recorded from all sampling plots using eTREX 20 (Garmin, Kansas, USA). Water physio-chemical parameters were measured following Baird et al. (2017). Ammonia, dissolved oxygen (mg/l), temperature (°C), electric conductivity (µS/cm), pH, and total dissolved solids (mg/l) were measured on site using a ProDSS multiparameter digital water quality meter (YSI, Ohio, USA). Water samples were collected and preserved at 5 °C for analysis of salinity (ppm) and turbidity (NTU). Salinity was measured using a salinity meter and turbidity was measured following a nephelometric method at the Soil Air and Water Testing (SWAT) laboratory of the College of Natural Resources (CNR), Royal University of Bhutan (RUB).

Species identification, collection, and preservation

Species were counted, photographed, and identified up to species level on site, then released back into the river. For specimens whose species identification was not possible on site, specimen color was noted while fresh, followed by euthanasia using 0.2 ml of clove oil per 500 ml of water and treatment with 10% formaldehyde solution (Fernandes et al., 2017; Arunkumar, 2020). The fixed specimens were preserved in 70% ethyl alcohol and cataloged at the Ichthyology Laboratory, CNR, RUB. The species were identified by comparing with known species from Bhutan and adjacent areas (Biswas et al., 2007; Vishwanath et al., 2007; Shrestha, 2008; Jayaram, 2010; National Research and Development Center for Riverine and Lake Fisheries, 2020). The methodologies and conventional procedures adhered to the principles outlined by Jayaram (2010). All measurements were made with a digital caliper to the nearest 0.1 mm and expressed as percentage of standard length (SL). Additionally, for diagnostics, meristic counts were performed following Armbruster (2012).

Taxonomic nomenclature

The nomenclature for all taxa mentioned in this study follows the updated classification provided by Fricke et al. (2023). This source was chosen due to its comprehensive and current coverage of the taxa under investigation. All species names and higher taxonomic ranks were checked and verified against this reference to ensure accuracy and consistency.

Data analysis

Initial data were entered, cleaned, and sorted using Microsoft Excel 2019 followed by descriptive analysis and computation of the relative abundance of cyprinid species. Given that there is no single diversity index that is universally considered as more suitable than others, six commonly used diversity indices were tested (Morris et al., 2014; Kelzang et al., 2021), namely Shannon diversity index (Shannon and Wiener, 1948), Simpson diversity index (Simpson, 1949), Pielou's evenness index (Pielou, 1966), Margalef's richness index (Margalef, 1958), Menhinick's index (Menhinick, 1964), and Sørensen's similarity coefficient (Sørensen, 1948).

The Kruskal—Wallis H test was conducted to assess the difference in diversity between the river basins, followed by Dunn's post-hoc test. Before analyzing the data, environmental variables were square-root transformed and cyprinid abundance data was $\log_{10}$ (x+1) for normalization and to meet the assumption of multivariate normality, as well as to moderate the influence of extreme data (Guo et al., 2018). Pearson's correlation was performed to examine the relationships between environmental variables. Canonical correspondence analysis (CCA) was conducted between cyprinid abundance (as the main matrix) and environmental parameters (as the secondary matrix) using PC-ORD v5.1 (ter Braak and Verdonschot, 1995; Grandin, 2006). Pearson correlation coefficients (r) were employed to assess the strength of correlation between variables along the axes. Monte Carlo simulation was performed to assess the significance of species—environmental parameter correlations (ter Braak and Verdonschot, 1995). Cluster analysis was carried out using Ward's method as a linkage method and the Euclidean matrix as the dissimilarity distance measurement (Ramírez et al., 2018). Indicator species in each cluster were determined using Monte Carlo simulation.

Results and Discussion

Summary of environmental variables and species

The resultant species–area curve suggests that the sampling efforts were adequate to characterize Cyprinidae composition in the study area (Fig. 2). A total of 11 environmental variables were collected and analyzed from 54 plots across four basins. However, only the mean values for each environmental variable are presented for each basin (Table 1). Similarly, a total of 850 individuals representing 22 species and 13 genera were recorded from the basins (Fig. 3). Among these species, one is categorized as Endangered (EN), two as Near Threatened (NT), two as Vulnerable (VU), one is listed as Data Deficient (DD), five species have not been assessed (NA), and eleven species are categorized as Least Concern (LC) (Table 2). *Neolissochilus hexagonolepis* was the most abundant species (n= 206), followed by *Schizothorax richardsonii* (n= 111), while *Garra lamta* was the least abundant species (n= 4). Similar observations were reported from the Himalayan region where *N. hexagonolepis* and *S. richardsonii* were the most dominant species (Dey et al., 2021).

Diversity and richness of cyprinids

The average species richness in Punatsangchhu, Aiechhu, Jaldakachhu, and Mangdechhu was 5.62±1.65, 5.89±1.54, 3.10±2.51, and 2.30±1.77, respectively. Aiechhu exhibited the highest diversity, as indicated by both the Shannon diversity index H' (1.66±0.28) and Simpson diversity index D (0.83±0.10), followed by Punatsangchhu (H'= 1.58±0.34, D= 0.83±0.10). The basins also demonstrated the highest species evenness with Pielou's evenness index (J') of 0.95±0.03 and 0.94±0.04, respectively, signifying not only a diverse but also a relatively even distribution of species. Similarly, Aiechhu's high species richness was supported by Margalef's diversity index (D_{Mg}) (5.45±1.56) and Menhinick's index (D_{Mn}) (1.42±0.30), followed by Punatsangchhu ($D_{Mg=}$ 5.24±1.68, D_{Mn}= 1.39±0.34). Such elevated species richness and diversity in the basins can be attributed to constant water flow and less modification of land (Kelzang et al., 2021). Discrepancies in reported cyprinid species exist between the current study and previous works. Tenzin (2022) reported eight cyprinid species from Aiechhu, whereas the current study recorded sixteen species. Similarly, Royal Society for Protection of Nature (2022) reported sixteen cyprinid species from Punatsangchhu, while the current study recorded twenty species. Such discrepancy can be attributed to variation in sampling method and temporal dynamics (Dey et al., 2021).

Contrarily, Jaldakachhu and Mangdechhu exhibited relatively lower values for diversity indices (Table 3). The findings are consistent with Wangchuk et al. (2017) and Royal Society for Protection of Nature (2022) who reported three and eight cyprinid species, respectively, from the Mangdechhu basin. The observed decline in species diversity and richness in these basins is attributed to their predominant flow through elevated terrain, river fluctuation, and potential anthropogenic pressure in the study area (Wangchuk et al., 2017; Simoes et al., 2021; Sor et al., 2023). Additionally, transboundary rivers experience a decrease in species diversity due to overfishing and pollution; such areas are emerging as key hotspots for species loss (Dolezsai et al., 2015; UNEP-DHI and UNEP, 2016).

Table 1: Summary of environmental variables in the River basins of this study.

Environmental variables	Aiechhu	Jaldakachhu	Mangdechhu	Punatsangchhu
Elevation (m asl)	313.60±103.22	680.30±261.78	1216.50±513.72	327.71±151.84
Turbidity (NTU)	2.96±1.29	2.12±1.36	6.07±6.72	9.49±12.12
pH	7.86±0.39	7.71±0.17	7.98±0.33	7.97±0.26
Temperature (°C)	25.75±1.49	18.73±2.99	15.22±4.65	23.96±2.46
Conductivity (µS/cm)	168.40±69.56	34.90±8.31	82.70±40.79	122.46±105.94
DO (mg/l)	8.19±0.45	10.09±1.35	6.31±2.50	8.68±0.58
TDS (mg/l)	77.19±32.41	16.16±4.61	54.30±28.75	57.13±50.94
Salinity (ppm)	86.65±36.39	15.64±1.98	58.23±31.84	62.95±56.96
Chloride (mg/l)	45.91±9.30	53.54±23.61	44.17±23.51	68.65±26.52
Ammonia (mg/l)	0.01±0.00	0.01±0.00	0.01±0.00	0.01±0.00
Total Hardness(mg/l)	57.45±15.63	44.60±19.00	14.90±11.86	60.52±16.14

Table 2: Cyprinid distribution (Presence/Absence) in the Aiechhu, Jaldakachhu, Mangdechhu, and Punatsangchhu River basins, with species identified by scientific name, species code, and their IUCN conservation status.

Species	Species code	IUCN status	Aiechhu	Jaldakachhu	Mangdechhu	Punatsangchhu
Bangana dero	Bade	LC	+	+	−	+
Semiplotus semiplotus	Cyse	VU	+	+	−	+
Esomus danrica	Esda	LC	+	−	−	−
Garra arupi	Gaar	NA	+	+	+	+
Garra annandalei	Gaan	NA	+	+	+	+
Garra birostris	Gabi	LC	+	+	+	+
Garra lamta	Gala	LC	−	−	−	+
Garra lissorhynchus	Gali	LC	−	−	+	−
Garra quadratirostris	Gaqu	NA	+	+	−	+
Labeo pangusia	Lapa	NT	+	+	−	+
Neolissochilus hexagonolepis	Nehe	NT	+	+	+	+
Opsarius barna	Baba	LC	+	+	+	+
Opsarius bendelisis	Babe	LC	+	+	+	+
Opsarius vagra	Bava	LC	+	−	−	+
Oreichthys crenuchoides	Orce	DD	+	−	−	+
Pethia conchonius	Peco	LC	+	−	+	+
Pethia ticto	Peti	LC	−	−	−	+
Puntius sophore	Puso	LC	+	+	−	+
Schizothorax progastus	Scpr	LC	−	+	+	+
Schizothorax richardsonii	Scri	VU	−	+	+	+
Tariqilabeo latius	Crla	LC	−	−	−	+
Tor putitora	Topu	EN	+	+	+	+

Note: += Present, −= Absent, LC= Least Concern, VU= Vulnerable, NA= Not Assessed, NT= Near Threatened, DD= Data Deficit, EN= Endangered.

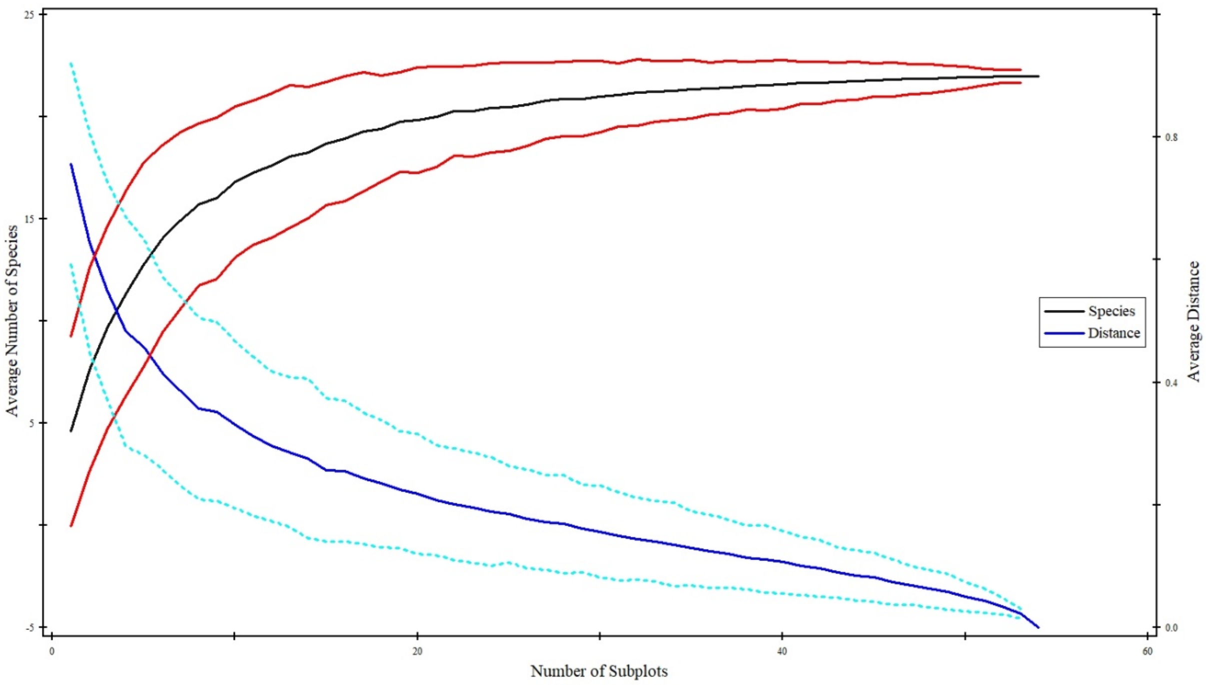

Figure 2: Species–area curve illustrating the correlation between number of plots and average species richness, based on 54 plots sampled. The observed stabilization point suggests adequate species sampling within the study area.

Figure 3: Cyprinids found in the study area, (A) *Bangana dero*, (B) *Semiplotus semiplotus*, (C) *Esomus danrica*, (D) *Garra arupi*, (E) *Garra annandalei*, (F) *Garra birostris*, (G) *Garra lamta*, (H) *Garra lissorhynchus*, (I) *Garra quadratirostris*, (J) *Labeo pangusia*, (K) *Neolissochilus hexagonolepis*, (L) *Opsarius barna*, (M) *Opsarius bendelisis,* (N) *Opsarius vagra*, (O) *Oreichthys crenuchoides*, (P) *Pethia conchonius*, (Q) *Pethia ticto*, (R) *Puntius sophore*, (S) *Schizothorax progastus*, (T) *Schizothorax richardsonii*, (U) *Tariqilabeo latius*, and (V) *Tor putitora.*

Table 3: Species richness and diversity indices of cyprinid populations in Aiechhu, Jaldakachhu, Mangdechhu, and Punatsangchhu River basins.

Basin	TS_R	S_R	H'	J'	D	D_{Mg}	D_{Mn}
Aiechhu	16	5.89±1.54	1.66±0.28	0.95±0.03	0.84±0.06	5.45±1.56	1.42±0.30
Jaldakachhu	14	3.10±2.51	0.90±0.66	0.84±0.54	0.51±0.30	2.68±2.57	0.83±0.48
Mangdechhu	9	2.30±1.77	0.54±0.70	0.38±0.49	0.34±0.44	1.71±2.03	0.61±0.18
Punatsangchhu	20	5.62±1.65	1.58±0.34	0.94±0.04	0.83±0.10	5.24±1.68	1.39±0.34

Note: $TS_{R=}$ Total species richness, S_R= average species richness, H'= Shannon diversity index, J'= Pielou's evenness index, D= Simpson diversity index, D_{Mg}= Margalef's richness index, D_{Mn}= Menhinick's index.

Sørensen's similarity coefficient among basins

To determine the similarity of species richness between the basins, Sørensen's similarity coefficient (CC) was examined (Table 4). Punatsangchhu and Mangdechhu had the highest similarity (64%), highlighting substantial overlap in species composition due to potential ecological similarities (Pavoine and Ricotta, 2014). Aiechhu exhibited a higher degree of similarity with both Mangdechhu (57%), and Punatsangchhu (56%), further highlighting commonalities in species composition. The lower similarity coefficients between Punatsangchhu and Jaldakachhu (47%), Aiechhu and Jaldakachhu (40%), and Jaldakachhu and Mangdechhu (32%) suggest a reduced overlap in species richness between these pairs of basins, indicative of a significant faunal break (Matthews, 1986; Radinger et al., 2016). This variation in similarity coefficients highlights the heterogeneity of species diversity patterns among the basins (Thomsen et al., 2022). Furthermore, these findings contribute to a comprehensive understanding of the conservation implications and priorities for cyprinids in river basins across Bhutan (Baselga, 2010).

Species diversity among river basins

The Kruskal—Wallis H test revealed significant differences in cyprinid composition among the river basins, χ^2 (3)= 20.98, $p < 0.001$. Pairwise comparison using Dunn's post-hoc test indicated significant difference in cyprinid diversity between Mangdechhu and Punatsangchhu (Z= 3.80, p= 0.00), Mangdechhu and Aiechhu (Z= 3.35, p= 0.01), and Jaldakachhu and Punatsangchhu (Z= 2.83, p= 0.03). However, no differences in cyprinid diversity were observed between Mangdechhu and Jaldakachhu, Jaldakachhu and Aiechhu, and Punatsangchhu and Aiechhu. Pathak et al. (2014) did show differences in species diversity among rivers in India, but their analyses showed these to be insignificant. While no previous studies have directly compared fish diversity, including cyprinids, among river basins in Bhutan, habitat heterogeneity is emerging as crucial factors influencing diversity (Cheng et al., 2019). Notably, the present study area exhibited habitat heterogeneity (Table 1). Low elevation basins (Punatsangchhu and Aiechhu) had favorable water parameters, while high elevation basins (Jaldakachhu and Mangdechhu) exhibited less favorable water parameters. Furthermore, larger habitats with greater a number of tributaries have numerous microhabitats, favorable environmental conditions, and food sources, supporting higher species diversity (Cheng et al., 2016). Conversely, smaller and disturbed habitats tend to have lower diversity (Kautza and Sullivan, 2012). The observed variations in cyprinid diversity within the study area highlight significant ecological distinctions, emphasizing the influence of habitat heterogeneity, environmental gradients, and human activities at both local and regional scales (Benke et al., 2011; Nicol et al., 2017; Sehr and Keckeis, 2017; Shukla and Bhat, 2017).

Pearson's correlation between environmental variables

Pearson's correlation between the environmental variables revealed that elevation was negatively correlated with temperature (r= −0.81, p= 0.00), conductivity (r= −0.48, p= 0.00), total dissolved solids (r= −0.39, p= 0.00), salinity (r= −0.34, p= 0.01), and total hardness (r= −0.59, p= 0.00), indicating an inverse relationship between elevation and these variables. Turbidity had moderate positive correlation with pH (r= 0.39, p= 0.00), and weak positive correlation with chloride (r= 0.30, p= 0.02) and total hardness (r= 0.29, p= 0.03). Temperature had moderate positive correlation with conductivity (r= 0.49, p= 0.00), total hardness (r= 0.45, p= 0.00), total dissolved solids (r= 0.36, p= 0.00), salinity (r= 0.35, p= 0.01), and weak negative correlation with dissolved oxygen (r= −0.36, p= 0.00). pH had moderate positive correlation with salinity (r= 0.43, p= 0.00), and weak correlation with conductivity (r= 0.49, p= 0.00) and total dissolved solids (r= 0.31, p= 0.01). Likewise, conductivity had strong positive correlation with salinity (r= 0.91, p= 0.00) and total dissolved solids (r= 0.88, p= 0.00), moderate positive correlation with total hardness (r= 0.33, p= 0.01), and weak negative correlation with dissolved oxygen (r= −0.27, p= 0.01). Dissolved oxygen had weak positive correlation with total hardness (r= 0.35, p= 0.01), and weak negative correlation with total dissolved solids (r= −0.38, p= 0.00) and salinity (r= −0.32, p= 0.00). There was a strong positive correlation between total dissolved solids and salinity (r= 0.83, p= 0.00). However, there was no correlation between chloride, ammonia, and total hardness (Table 5).

The observed correlations align not only with prior findings in the Himalayan region but also with studies conducted globally (Lakra et al., 2010; Dong et al., 2015; Al Dahaan et al., 2016; Borowia et al., 2020; Yavuzatmaca, 2020; Kothari et al., 2021; Thomas, 2021; Saalidong et al., 2022; Chaya et al., 2023; Dewangan et al., 2023). The negative correlation of water with environmental variables aligns with ecological principles where higher elevation is often associated with cooler temperatures and lower mineral content in water (Ghobadi et al., 2018). The moderate positive correlation between turbidity and pH and the weak correlation between turbidity and chloride, along with total hardness, signifies a higher pH level and concentration of ions. The strong positive relation between temperature and conductivity, total hardness, total dissolved solids, and salinity indicates higher salt and mineral content in water, potentially favoring reproduction, habitat expansion, and metabolism (Martemyanov and Borisovskaya, 2012). However, these factors can also impact osmoregulation and disrupt food web dynamics (Dornelas et al., 2020). The pH and conductivity correlation underscores their interconnectedness. This correlation is potentially indicative of shifts in salinity. The weak negative association between temperature and dissolved oxygen and the positive correlation between dissolved oxygen and total hardness indicate that water temperature can contribute to lower dissolved oxygen levels (Thomas, 2021), impacting aquatic organisms. The strong relationship between total dissolved solids and salinity highlights that salt is a major component of dissolved solids (Maliki et al., 2020).

Table 4: Sorenson's similarity coefficient (CC) matrix showing the similarity of species richness between Aiechhu, Jaldakachhu, Mangdechhu, and Punatsangchhu River basins, highlighting potential ecological similarities and faunal breaks.

	Aiechhu	Jaldakachhu	Mangdechhu	Punatsangchhu
Aiechhu	1.00	0.40	0.57	0.56
Jaldakachhu		1.00	0.32	0.47
Mangdechhu			1.00	0.64
Punatsangchhu				1.00

Table 5: Correlation matrix of environmental variables in the study area, showing Pearson's correlation coefficients between 11 environmental variables collected from Aiechhu, Jaldakachhu, Mangdechhu, and Punatsangchhu River basins.

	Elev	Tur	pH	Tem	Con	DO	TDS	Sal	Ch	Am	TH
Elev	1.00										
Tur	−0.15	1.00									
pH	−0.06	0.39**	1.00								
Tem	−0.81**	0.06	0.00	1.00							
Con	−0.48**	0.24	0.36**	0.49**	1.00						
DO	0.16	0.12	−0.15	−0.36**	−0.27*	1.00					
TDS	−0.39**	0.13	0.31*	0.36**	0.88**	−0.38**	1.00				
Sal	−0.34*	0.21	0.43**	0.35**	0.91**	−0.32*	0.83**	1.00			
Ch	−0.15	.030*	−0.11	0.21	−0.06	0.02	−0.09	−0.10	1.00		
Am	0.08	−0.10	−0.14	−0.10	−0.06	0.11	−0.18	−0.09	0.02	1.00	
TH	−0.59**	0.29*	0.05	0.445**	0.33*	0.35*	0.17	0.26	0.22	0.20	1.00

Note: ** and * correlation significant at the 0.01 and 0.05 level, respectively. N= 54; Note: Elev= Elevation, Tur= Turbidity, Tem= Temperature, Con= Conductivity, DO= Dissolved oxygen, TDS= Total dissolved solids, Sal= Salinity, Ch= Chloride, Am= Ammonia, TH= Total hardness.

Effect of environmental variables on Cyprinidae assemblage

Canonical correspondence analysis (CCA) was performed between Cyprinidae abundance and environmental variables. The analysis included 11 environmental variables and 22 cyprinid species. Axis 1 (54%) and Axis 2 (23%) explained 77% of the variance in a species—environmental relationship (Fig. 4). The species—environment correlation coefficient for axes 1 and 2 were 0.94 and 0.82, respectively (Table 6). Monte Carlo simulation revealed that cyprinid assemblage was significantly correlated with environmental variables (r= 0.94, p= 0.001 in Axis 1 and r= 0.82, p= 0.001 in Axis 2). Axis 1 was strongly positively correlated with temperature (r= 0.92), moderately correlated with total dissolved solids (r= 0.53) and conductivity (r= 0.51), and weakly correlated with salinity (r= 0.39), and negatively correlated with elevation (r= −0.69) and dissolved oxygen (r= −0.24). Similarly, there was a strong to weak positive correlation between *Neolissochelius hexagonolepis* (r= 0.55), *Garra annandalei* (r= 0.50), *Garra birostris* (r= 0.38), *Opsarius barna* (r= 0.36), *Opsarius bendelisis* (r= 0.35), and *Tariqilabeo latuce* (r= 0.35) with the Axis 1. Some cyprinids exhibited positive affinity towards water temperature, such as *O. barna*, *O. vagra* and *Schizothorax plagiostomus*, while others, including. *S. richardsonii*, exhibited a strong negative correlation (r= −0.93), highlighting species specific adaption to environmental conditions (Limbu et al., 2020). This species-specific adaption potentially arises from a combination of ecological factors including feeding behavior, physiological tolerances, habitat preferences, and morphological characteristics (Ward-Campbell et al., 2005).

Similarly, Axis 2 demonstrated strong negative correlation with total hardness (r= −0.82) and dissolved oxygen (r= −0.65). However, ammonia, chloride, turbidity, and pH had minimal influence on species assemblage and were consequently excluded from the ordination biplot. The position of cyprinid species in relation to environmental variables is shown in Figure 5.

The effect of elevation on distribution and assemblage of cyprinids is corroborated by several studies (Jaramillo-Villa et al., 2010; Carvajal-quintero et al., 2015; Rajbanshi et al., 2021; Soo et al., 2021). Cyprinid assemblage is effected by small scale altitudinal gradients (Soo et al., 2021). Wangmo and Rai (2019) highlighted that cyprinids usually prefer lower elevations with warmer water, dominating these habitats across the Himalayan region. This preference for lower elevation is reflected in the present study with occurrence and composition of cyprinids decreasing as elevation increased, elucidating the influence of elevation on cyprinid assemblage. Cheng et al. (2019) noted a decrease in alpha diversity of cyprinids with increased altitude, reflecting changing environmental conditions. Above 600 m asl, cyprinids are replaced by Gastromyzontidae (Nyanti et al., 1995; Soo et al., 2021), highlighting the distinct ecological dynamics at higher elevations.

Temperature is directly related to several physiological processes, including reproduction, and has a direct relationship with cyprinid species diversity and assemblage. Hu et al. (2019) underscored that temperature has a significant effect on cyprinid distribution and assemblage composition. The presence of cyprinids such as *Pethia ticto*, *Puntius sophore*, and *Esomus danrica* was positively related to temperature. This finding is consistent with observations made by Chaudhary and Limbu (2021), who also reported a strong positive correlation between cyprinids and temperature. Cyprinids are generally warm-adapted fish and prefer temperatures between 13 °C to 18.2 °C with an optimum temperature of 15 °C (Graham and Harrod, 2009; Wangmo and Rai, 2019). However, there are few cyprinids, such as *S. richardsonii*, that can survive at lower temperature (Li et al., 2012; Gurung and Thoni, 2015).

Similarly, Pokharel et al. (2018) highlighted that cyprinid species including *Neolissochelius hexagonolepis*, *Garra annandalei*, and *G. birostris* prefer positively correlated values of conductivity and total dissolved solids. An increased concentration of total dissolved solids and electrical conductivity can disrupt osmoregulation, leading to stress and mortality in cyprinids (Adjovu et al., 2022). Similarly, pH value is an essential environmental factor influencing the assemblage of freshwater cyprinids and should typically range between 6.5 to 9.0. Beyond this range, cyprinids face survival challenges, necessitating migration to more favorable conditions (Scott et al., 2005; Wangmo and Rai, 2019). Despite the crucial role of pH, the current study did not find any correlation between cyprinid assemblage and pH. However, some cyprinids such as *Opsarius barna*, *O. vagra*, and *Labeo* spp. are positively related to pH and dissolved oxygen (Chaudhary et al., 2020). Conversely, Shrestha et al. (2021) reported pH at a trace level as a significant factor for cyprinid distribution. Such discrepancy underscores the complexity of cyprinid ecology and the need for further research to elucidate the role of pH in species distribution.

Additionally, the present study observed a negative relationship between cyprinid assemblage and dissolved oxygen. Pokharel et al. (2018) underscored that cyprinids preferred negatively correlated values of dissolved oxygen. The observed preference for warmer water conditions despite lower dissolved oxygen levels suggests adaptability of cyprinids to varying environmental conditions, possibly driven by migration to habitats with optimal conditions (Shrestha et al., 2021). Additionally, cyprinids are characterized by their high tolerance to pollution, lower dissolved oxygen concentration, and their abundance in warmer waters (Shen et al., 2016). This trend is evident in the current study, where lower dissolved oxygen levels were associated with a higher abundance of cyprinids, suggesting a preference for warm water conditions.

Table 6: Summary of CCA and Monte Carlo stimulation of cyprinid abundance and environmental variables.

Measures	Axis 1	Axis 2
Eigen value	0.7	0.16
Species environmental correlation coefficient	0.94	0.82
% of variance explained	54	23
Significance of correlation (Monte Carlo)	0.024	0.02
Significance of Eigen value (Monte Carlo)	0.001	0.001

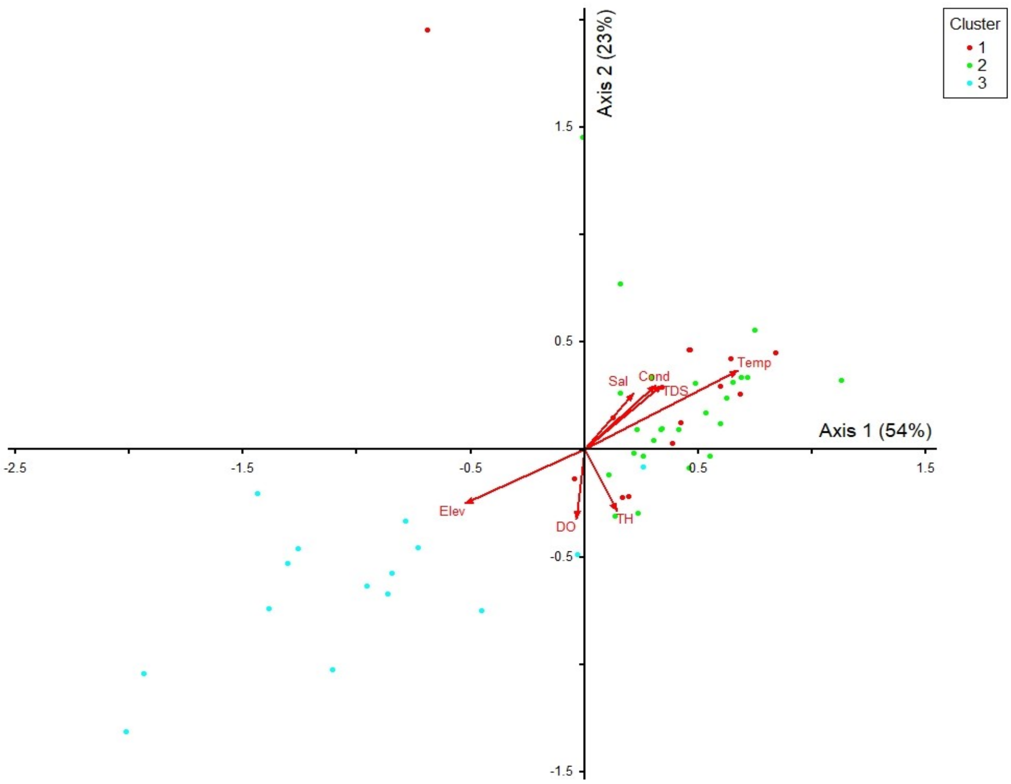

Figure 4: CCA ordination of cyprinid species in relation to environmental variables.

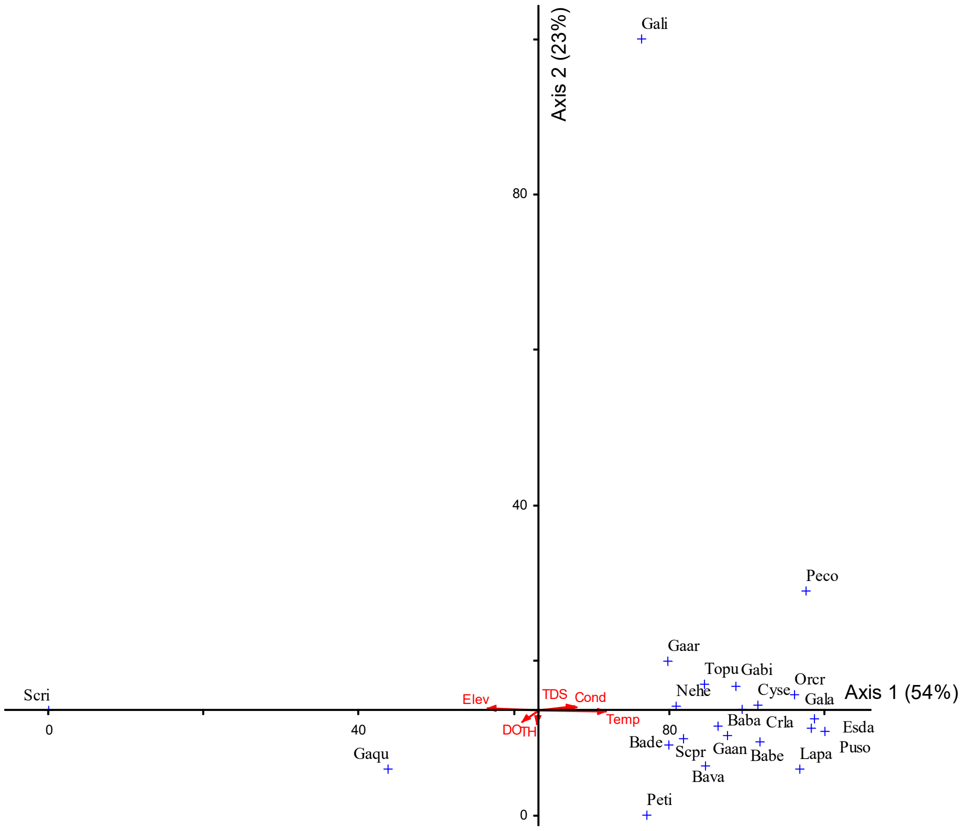

Figure 5: CCA ordination of cyprinid species. For species code see Table 2.

Cluster analysis

Cluster analysis using Ward's method as a linkage method and the Euclidean matrix as the dissimilarity distance measurement resulted in three distinct clusters at 50% similarity index (Fig. 6). The naming of the cluster was done based on dominant and indicator species and the most influential environmental variables obtained from the CCA biplot.

Cluster One (Temperate Garra Cluster)

In this cluster, a total of 14 sampling plots (Fig. 6) were consolidated, with an average elevation of 402.5±200.18 m asl and an average temperature of 22.87±3.06 °C. The dominant species identified, including *Esomus danrica*, *Tariqilabeo latius*, *Garra arupi*, *G. annandalei*, *G. birostris*, and *G. lissorhynchus*, are typically associated with the transition from warm to temperate water (Shrestha et al., 2021). Indicator species analysis using Monte Carlo simulation further highlighted *G. arupi*, *G. annandalei*, and *G. birostris* as indicator species in this cluster ($p < 0.01$). Notably, according to Thoni et al. (2016), these species predominantly thrive in mid-elevation streams throughout Bhutan.

Cluster Two (Warm Mahseer Cluster)

Twenty-five plots were consolidated into this cluster (Fig. 6). The prominent environmental features included a relatively lower elevation of 322.92±135.40 m asl and relatively warm water at 27.23±2.03 °C, facilitating the survival of these cyprinids. The most dominant species in this cluster was *Neolissochelius hexagonolepis*, classified as Near Threatened, but abundant in all river basins across Bhutan (Gurung and Thoni, 2015), followed by *Opsarius barna*. The indicator species in the cluster included *O. barna*, *O. bendelisis*, *Tor putitora*, *Semiplotus semiplotus*, *O. vagra*, *Pethia conchonius*, *N. hexagonolepis*, and *Oreichthys crenuchoides* ($p < 0.01$). These species are typically found dwelling in warm water across the Himalayan region (Dorji and Gurung, 2017; Gurung et al., 2013; Shrestha, 2008).

Cluster Three (Cold Trout Cluster)

This cluster consisted of 15 plots. The cluster had an average elevation of 1,095.07±489.60 m asl and mean water temperature of 15.59±3.82 °C. *Schizothorax richardsonii* and *Garra quadratirostris* were the most dominant species in these plots. Wangmo et al. (2023) highlighted that *S. richardsonii* is found dwelling up to 5,000 m asl in Bhutan. Similarly, Gurung and Thoni (2015) and Thoni et al. (2016) highlighted that *G. quadratirostris* is found up to 1,000 m asl across all river basins in Bhutan. The indicator species analysis identified *S. richardsonii* as indicative of this cluster ($p < 0.01$), consistent with the findings of Kelzang et al. (2021) and Dey et al. (2021) where the species was observed dominating cold waters across its native habitat.

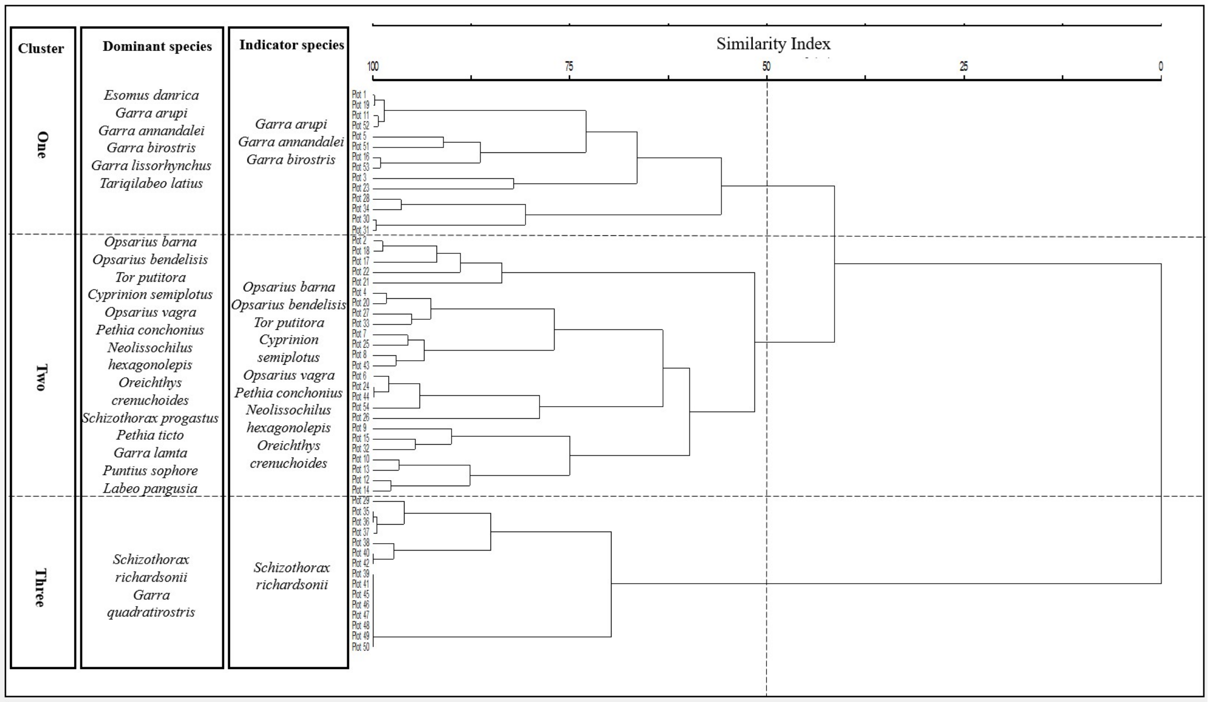

Figure 6: Cluster dendrogram of 54 sampling sites, depicting a 50% similarity index along with dominant and indicator species.

Conclusion

The present study sheds light on the diversity and assemblage of cyprinids across four river basins in Bhutan. *Neolissochilus hexagonolepis* emerged as the most abundant species, closely followed by *Schizothorax richardsonii*, with *Garra lamta* being the least abundant. The diversity and richness of cyprinids varied among the basins with Aiechhu exhibiting the highest diversity and even distribution of species. Sørensen's similarity coefficient highlighted substantial overlaps in species composition between Punatsangchhu and Mangdechhu, while significant faunal breaks were observed between other pairs of basins. Canonical correspondence analysis (CCA) elucidated the intricate relationship between cyprinid abundance and environmental variables, with elevation, temperature, dissolved oxygen, total dissolved solids, and conductivity emerging as key influencers. The cluster analysis identified three distinct clusters—Temperate *Garra*, Warm Mahseer, and Cold Trout—based on dominant and indicator species and influential environmental variables. This clustering provides a practical framework for understanding the ecological niches and habitat preferences of cyprinids within the studied basins. In light of these findings, it is recommended to conduct further studies exploring other river basins across Bhutan, incorporating a larger number of sampling plots and an expanded set of environmental variables. The impact on conservation strategies emphasizes the need for basin-specific approaches, tailored to the unique ecosystems identified in this study. The dominance of cyprinids in Bhutan's river basins, underscores the vital call for biodiversity conservation and there is a pressing need for a standardized cyprinid conservation approach.

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Author contributions

L. S., K. L. W., R. D., T. Z., and R. S. designed the study and conducted the field sampling. L. S., D. B. G., and K. W. analyzed the data and wrote the manuscript draft. L. S. developed the final article.

Conflict of interest

The authors declare that there are no conflicting issues related to this research article.

Ethical standards

This research project with permit number 156186516462BAB1DFAB732 was approved by Ugyen Wangchuk Institute for Forestry Research and Training, Lamaigoenpa, Bumthang, Bhutan. Specimen collection and preservation were done following the standard protocol as reflected in the method section and in accordance with the Forest and Nature Conservation Rules and Regulations (FNCRR), 2017 and the Biodiversity Monitoring and Social Survey Protocol of Bhutan, 2020.

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