Spatiotemporal Variation of Fish Assemblages in Triyuga River, Eastern Nepal

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A dissertation submitted
In partial fulfillment of the requirements for the award of the degree of Master of Science in Zoology with special paper Fish Biology and Aquaculture

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Declaration

I hereby declare that the work presented in this dissertation **"Spatiotemporal Variation of Fish Assemblages in Triyuga River, Eastern Nepal"** has been done by myself, and has not been submitted elsewhere for the award of any degree. All sources of information have been specifically acknowledged by reference to the author(s) or institution(s).

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On the recommendation of supervisor "Associate Professor Dr. Archana Prasad" this dissertation submitted by Ajay Jha entitled **"Spatiotemporal Variation of Fish Assemblages in Triyuga River, Eastern Nepal"** is approved for the examination in partial fulfillment of the requirements for Master's Degree of Science in Zoology with special paper 'Fish Biology and Aquaculture'.

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Certificate of acceptance

This dissertation work submitted by Ajay Jha entitled **"Spatiotemporal Variation of Fish Assemblages in Triyuga River, Eastern Nepal"** has been accepted as a partial fulfillment for the requirements of Master's Degree of Science in Zoology with special paper 'Fish Biology and Aquaculture'.

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Abstract

This study investigates the spatiotemporal variations in fish diversity and assemblage structure in the Triyuga River, located in Saptari District, Nepal. Sampling was conducted from June to December 2024 across five stations namely Phatteypur (I), Kamalpur (II), Pathari (III), Bairwa (IV), and Koshi Baan (V) covering parts of Saptakoshi, Kanchanrup, and Hanumannagar Kankalini Municipalities. Fish were collected using a cast net of mesh size (1×1) cm^2 and identified using standard taxonomic keys (Jayaram, 2010; Shrestha, 2019). A total of 32 fish species were recorded, representing 7 orders, 15 families, and 25 genera, with Cypriniformes being the most dominant order (47%). *Pachypterus atherinoides* (Siluriformes) emerged as the most abundant species (22.74%), while *Anabas cobajius*, *Opsarius shacra*, *Rasbora daniconius*, *Raiamas bola*, and *Xenentodon cancila* were among the least represented (0.07%). Seasonal analysis revealed that the Shannon-Weiner index (H'), which measures species diversity, was lowest at Station IV (2.3) and summer (2.46), and highest in the fall (2.66), followed by Station III (2.6). The availability of food, steady water flow, and moderate temperatures are probably the main environmental factors causing these variances. Margalef's richness index had spatial values ranging from 2.84 at Station IV to 4.46 at Station I, with a seasonal peak in autumn (4.08) and a low in winter (3.41). Pielou's evenness was highest in the winter (0.84) and lowest in the summer (0.76), With a range of 0.73 at Station I to 0.85 at Station IV. Physicochemical analysis revealed that water temperature ranged from 17.9°C to 33.0°C, transparency varied from 89 cm at Station I to 37 cm at Station V, pH ranged between 7.7 and 8.5, dissolved oxygen (DO) levels ranged from 5.67 mg/L to 7.25 mg/L, and water hardness ranged from 47.0 mg/L to 59.06 mg/L. Redundancy Analysis (RDA) revealed that environmental variables particularly temperature, dissolved oxygen (DO), pH, and hardness had a significantly positive influence on the structure of fish assemblages. The findings highlight the ecological importance of the Triyuga River and underscore the need for ongoing monitoring and conservation strategies to protect aquatic biodiversity and ensure the sustainability of fishery resources in the region.

शोधसार

यस अध्ययनले सप्तरी जिल्लामा अवस्थित त्रियुगा नदीमा माछाको जैविक विविधता र समुदाय संरचनामा देखिने स्थानिक तथा मौसमी (Spatiotemporal) परिवर्तनहरूको विश्लेषण गरेको छ। नमूना सङ्कलन सन् २०२४ को जुन देखि डिसेम्बरसम्म फत्तेपुर (I), कमलपुर (II), पथरी (III), बैरवा (IV), र कोशी बाँन (V) गरी पाँचवटा स्टेशनहरूमा गरिएको थियो, जसले सप्तकोशी, कञ्चनरुप, र हनुमाननगर कङ्कालिनी नगरपालिकाका क्षेत्रहरू समेट्छ। नमुना संकलनका लागि १*१cm^2 को हाथेजाल र स्थानीय माझीको सहयोगबाट गरिएको थियो । संकलित माछाका नमुनाहरु Taxonomic keys [Jayaram, 2010 र Shrestha, 2019] को आधारमा पहिचान गरिएको थियो । यस अध्धयनमा ३२ प्रजाति, ७ अर्डर, १५ फ़ैमलिस र २५ जेनेराका माछाहरु संकलन गरिएको थियो, जसमध्ये Cypriniformes अर्डर सबैभन्दा बढी मात्रामा (४७%) पाइयो। *Pachypterus atherinoides* (Siluriformes) सबैभन्दा प्रचुर प्रजातिका रूपमा (२२.७४%) देखिएको थियो, जबकि *Anabas cobajius, Opsarius shacra, Rasbora daniconius, Raiamas bola*, र *Xenentodon cancila* न्यूनतम मात्रामा (०.०७%) प्रतिनिधित्व भएका थिए। मौसमी विश्लेषण अनुसार शरद ऋतुमा सबैभन्दा बढी प्रजाति विविधता (Shannon-Weiner index H' = २.६६) देखिएको थियो भने गर्मीयाममा सबैभन्दा कम (H' = २.४६) देखिएको थियो, जुन सामान्यतः मध्यम तापक्रम, स्थिर प्रवाह र प्रशस्त आहार उपलब्धतासँग सम्बन्धित थियो। Margalef को प्रजाति समृद्धि सूचक शरद ऋतुमा ४.०८ र जाडोमा ३.४१ थियो, जबकि Pielou को समानता सूचक जाडोमा सबैभन्दा उच्च (०.८४) र गर्मीयाममा सबैभन्दा न्यून (०.७६) थियो। पारिस्थितिक तत्त्वहरूको विश्लेषणमा पानीको तापक्रम १७.९°C देखि ३३°C,Transparency को मान स्टेशन I मा ८९ cm र स्टेशन V मा ३७ cm, pH मान ७.७ देखि ८.५, Dissolved Oxygen (DO) ५.६७ mg/L देखि ७.२५ mg/L, र पानीको Hardness ४७.० mg/L देखि ५९.०६ mg/L बीच रहेको पाइयो। Redundancy Analysis (RDA) बाट यी वातावरणीय कारकहरू–विशेषगरी तापक्रम, DO, pH र Hardness ले माछाको समुदाय संरचनामा महत्त्वपूर्ण रूपमा सकारात्मक प्रभाव पार्ने देखियो। अन्ततः, यस अध्ययनले त्रियुगा नदीको पारिस्थितिक महत्वलाई उजागर गर्दै, नदीको जैविक विविधता संरक्षण र दीगो मत्स्य स्रोत व्यवस्थापनका लागि निरन्तर अनुगमन र संरक्षण रणनीतिहरू आवश्यक रहेको संकेत गर्दछ।

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Abbreviated Form	Details of Abbreviation
APHA	American Public Health Association
DCA	Detrended Correspondence Analysis
EDTA	Ethylene Diaminetetraacetic Acid
mg/L	Miligram per litre
ppm	Parts per million
RDA	Redundancy Analysis
µS/cm	Microsiemens per centimeter

1. Introduction

1.1 Background of the study

There are around 35800 species of fish (Froese & Pauly, 2024), making them one of the most significant and diverse groups of vertebrates. Fish are closely linked to the welfare of humans (Lévêque et al., 2008). There are more than 3,000 fish species in Asia (Lundberg et al., 2000), and the most dominant freshwater fish in South Asia are carps (Cypriniformes) and catfishes (Siluriformes) (Berra, 2007; Nelson et al., 2016).

The fish community structure is shaped by physicochemical environmental factors, showing variations across different locations and over time, along with biotic interactions like competition and predation (Gorman, 1988; Harvey & Stewart, 1991; Grossman et al., 1998). Environmental factors are known to affect the spatial patterns of species distribution (Perry et al., 2005) and drive temporal changes in community composition (Rouyer et al., 2008). Stream width, elevation, and proximity to the source are some of the variables that affect the variety and composition of fish communities in different watercourses (Li et al., 2011). The location of catchments and habitat attributes like substrate type and water depth were linked to variations in spatial distribution (Arthington et al., 1993). Rivers are dynamic ecosystems because of their large and unpredictable changes in flow, temperature, dissolved oxygen, pH, and conductivity, among other physical and chemical properties. Fish community diversity and composition have been observed to be impacted by changes in river systems (Grossman, 1982). According to Bhatt (2012), the species richness of fish uniformly decreased with elevation.

Yap (2002) provides a historical perspective on the distribution of freshwater fish in Southeast and East Asia, highlighting the fact that the existence of these varied species is confirmed by evolutionary history and fossil evidence. Additionally, ecological factors, such as habitat changes and human activities, have been observed to impact fish diversity. Studies in Guangxi, China, show that environmental changes have influenced species composition (He *et al.*, 2022). Along with the species composition and structure in streams, springs, and rivers, fish variety is a major factor in how well fish are collected in waterways and springs. Geographical scales may cause these elements to vary (Negi & Mamgain, 2013). Compared to temperate regions, the structure and heterogeneity of fish communities are more prominently expressed in tropical riverine and waterway environments. However, there remains a lack of comprehensive information on tropical fish communities (Nair et al., 1989; Arunachalam et al., 1997; Arunachalam & Martin, 2000; Sankaranayanan, 1999).

The first publication, *Fishes of Nepal* (1981), established Shrestha as a pioneering indigenous ichthyologist. In this work, She described 120 species of freshwater fish from 63 genera, 10 orders, and 26 families. Since then, ichthyological research in Nepal has grown significantly. Shrestha (2001) reported 182 fish species, while Rajbanshi (2005) listed 187 species. Later, Saud and Shrestha (2007) identified 199 species, and Shrestha (2008) recorded 217 native species along with 15 exotic ones. Shrestha (2019) reported 236 native fish species and 16 exotic ones. Notably, the Koshi River in eastern Nepal is home to 200 fish species.

The Triyuga River, originating from Rautahapokhari in Okhreni (2,110 m) on the Mahabharat Range in Udayapur District, flows southward, fed by tributaries like Babiya, Lohale, Kalikhola, and Duwar, before merging with the Saptakoshi at Tapeswary in Saptari District. It is known for its rich fish diversity, making it ecologically and scientifically significant. Shrestha (2016) documented 48 fish species from the Triyuga River in eastern Nepal. Despite extensive research on major river systems, has been done but still there is a need to investigate medium and small-sized rivers that have not been studied in the past half decade. Therefore, this study focuses on exploring fish diversity in the Triyuga River.

1.2 Objectives

1.2.1 General objective

The general objective of this study is to assess spatiotemporal variation of fish assemblages in Triyuga River, Eastern Nepal.

1.2.2 Specific Objectives

- To assess the fish diversity and abundance.
- To find the diversity status using different diversity indices.
- To analyse physico-chemical parameters of water.

- To find out the relationship between environmental variables with fish species.

1.3 Significance of the study

- It has helped to assess the rivers biodiversity by identifying the different species, their abundance and distribution patterns over time and place.
- It has provided knowledge about resource management and conservation, assisting in securing the long-term survival of the fish populations and ecosystem of the Triyuga River.

1.4 Limitations of the Study

- Using a multi-parameter tool could have improved the testing of various additional water parameters like arsenic, chloride, nitrite which would have given precise information regarding the maintaining of both water quality and fish species.

2. Literature review

2.1 Spatial variation of fish assemblage structure

Fish assemblages are essential components of aquatic ecosystems and are considered reliable indicators of freshwater ecosystem health due to their sensitivity to a wide range of environmental stressors (Oberdorff et al., 1995). The study by Bailey and Ahmadi (2014) revealed that dissolved oxygen (DO) and nitrate (NO_3) concentrations in streams vary significantly across locations within a river network. Upstream solute concentrations, algal processes, groundwater discharge, and oxygen reaeration dominate DO and NO_3 patterns. However, the influence of these factors changes spatially along the river and tributaries, with groundwater impacts being more pronounced in tributaries. Fish assemblages vary along spatial gradients, particularly between upstream and downstream sites (Buisson & Grenouillet, 2009). Upstream regions are predicted to experience stronger species turnover, greater trait diversity increases, and even assemblage differentiation, while midstream and downstream areas are expected to homogenize due to colonization by generalist species. However, a major gap remains as models often assume universal dispersal, ignoring natural and artificial barriers like dams and watershed isolation, which could limit species movements (Buisson & Grenouillet, 2009). Fish assemblages in the Mary River showed clear spatial variation mainly due to habitat structure, particularly substrate composition, water depth, and cover availability, with differences strongly influenced by catchment position (Arthington et. al., 1993).

Spatial variation in fish assemblages is mainly driven by habitat characteristics such as depth, velocity, substrate type, and river discharge patterns (Li & Gelwick, 2005). In the Brazos River, variation among sites was stronger than seasonal changes, with deep-water and shallow-margin habitats supporting distinct fish communities. However, a key gap remains in understanding the finer-scale spatial use within large floodplain rivers, especially across dynamic interfaces like embankments and tributary confluences. Spatial variation in fish assemblages was strongly structured by large-scale habitat features such as distance to source, stream width, altitude, and water pH (Li et al., 2012). Fish species richness and diversity generally increased from upstream to downstream, with assemblage differences mainly associated with stream geo-morphology and anthropogenic impacts. However, existing studies still lack detailed understanding of how microhabitat complexity within reaches (like riffle-pool units) further shapes local fish assemblage patterns. Bertoni and Hued (2002) showed that fish species richness declines as elevation increases, and that overall diversity rises with the spatial extent of river networks in both temperate and tropical regions. Shrestha (2016) reported a higher ichthyofaunal diversity in Nepalese rivers, identifying 48 species across 35 genera and 17 families. Shrestha (2023), focusing on the Dudhkoshi River, recorded 22 species, among which *Puntius sophore*, *Opsarius shacra*, and *Garra annandalei* were also documented, indicating a broad ecological tolerance of these species across different river systems in Eastern Nepal. A survey by Prasad et al. (2020), reported the influence of key environmental variables like- pH, total hardness, alkalinity, dissolved oxygen, and water temperature, with the fish community in the Seti Gandaki River. They identified 46 species across three orders, nine families, and 23 genera.

2.2 Temporal variation of fish assemblage structure

Fish communities are influenced by both biotic and abiotic causes. Predation is one of the primary causes of ecological trends in freshwater fish groups. Resource distribution among fish populations plays a significant role in shaping community structure, with competition identified as a key ecological factor influencing composition (Gebrekiros, 2016). A fish assemblage's diversity and structure may be affected differently by climate change depending on where it is in the upstream-downstream gradient (Buisson and Grenouillet, 2009). Temporal variation was relatively low, with seasonality explaining only a small part of the changes in fish assemblages, compared to the stronger influence of habitat and flow conditions (Li & Gelwick, 2005). While broad seasonal patterns like juvenile recruitment and migration were observed, there is still limited knowledge on how fine-scale seasonal and life-stage movements shape assemblage structure, especially in response to irregular flood pulses.

Temporal variation was mainly driven by changes in cover elements like macrophytes and leaf litter, while major floods caused little structural change to habitats, indicating that short-term shifts were mostly related to shelter availability rather than physical disturbance (Arthington et. al., 1993). Bailey and Ahmadi (2014) found that seasonal changes, especially in water temperature and solar radiation, strongly affect biological and chemical processes such as algal growth, respiration, and oxygen aeration. These factors exhibit stronger influence during summer months compared to winter. However, the study did not fully address the impact of extreme events (e.g., floods, droughts) or inter-annual variability, leaving a temporal gap in understanding shorter-term or episodic variations.

Alkalinity, turbidity, and dissolved oxygen levels are all directly impacted by water temperature (Fisher and Willis, 2000). Chen et al. (2023) observed that interannual variation in fish biomass, species composition, and abundance was controlled by total suspended particles and dissolved oxygen. During the winter, lower temperatures lead to a reduction in dissolved oxygen levels in the water, while higher temperatures in the summer typically result in an increase in dissolved oxygen. The structure of fish assemblages in streams and rivers declines at higher elevations as a result of changes in water temperature (Jacobsen, 2008; Jaramillo-Villa et al., 2010). Seasonal variation in environmental and physicochemical conditions significantly influences the distribution and composition of fish assemblages in stream ecosystems (Beugly and Pyron, 2010). The Triyuga River's aquatic ecosystem is important for supporting many kinds of fish. Earlier studies, like those by Shrestha (2016) and Shakya et al. (2007), have shown that the river is a key habitat for both native and migratory fish species.Their findings suggested that substrate composition and aquatic vegetation significantly influence fish distribution and fish abundance. Yadav (2017) observed that fish diversity in the Sapta Koshi River varied significantly across different sampling stations, with transparency showing both positive and negative correlations with fish density depending on the location. Since transparency is influenced by flow conditions and sediment, these results imply the indirect role of water velocity in shaping species abundance. Comparison with the Koshi River provides a broader perspective on the findings. Saud (2011) documented 61 species belonging to 7 orders, 20 families, and 41 genera in the Koshi River within the Koshi Tappu Wildlife Reserve, indicating a higher overall species richness than that of the Triyuga River. Similarly, Shah (2016) noted that the Koshi River's diverse habitats including floodplains and oxbow lakes contribute significantly to supporting a higher ichthyofaunal diversity.

3. Materials and methods

3.1 Study area

The present study area, Triyuga River (26° 39'-27°22'N, 86° 9'-87°10' E) is located in Eastern Nepal. It is bordered by nine districts: Dhankuta and Sunsari in the east, Saptari and Siraha in the south, Dhanusa and Sindhuli in the west, and Okhaldhunga, Khotang, and Bhojpur in the north. The Triyuga River is a significant river in the district, with an approximate length of 42.5 km.

Initially, two small streams in the form of lake drainage emerge from two separate locations on the lake and run down to the south slope, where they confluence and become the river Triyuga. The river receives water from several streams on the plain and eventually flows to the Saptakoshi. Five (5) study sites were established to collect fish species from various sampling areas of the Triyuga River in the Saptari district viz, Site I-Phatteypur, Site II-Kamalpur, Site III-Pathari, Site IV-Bairwa and Site V-Koshi baan (Fig.1).

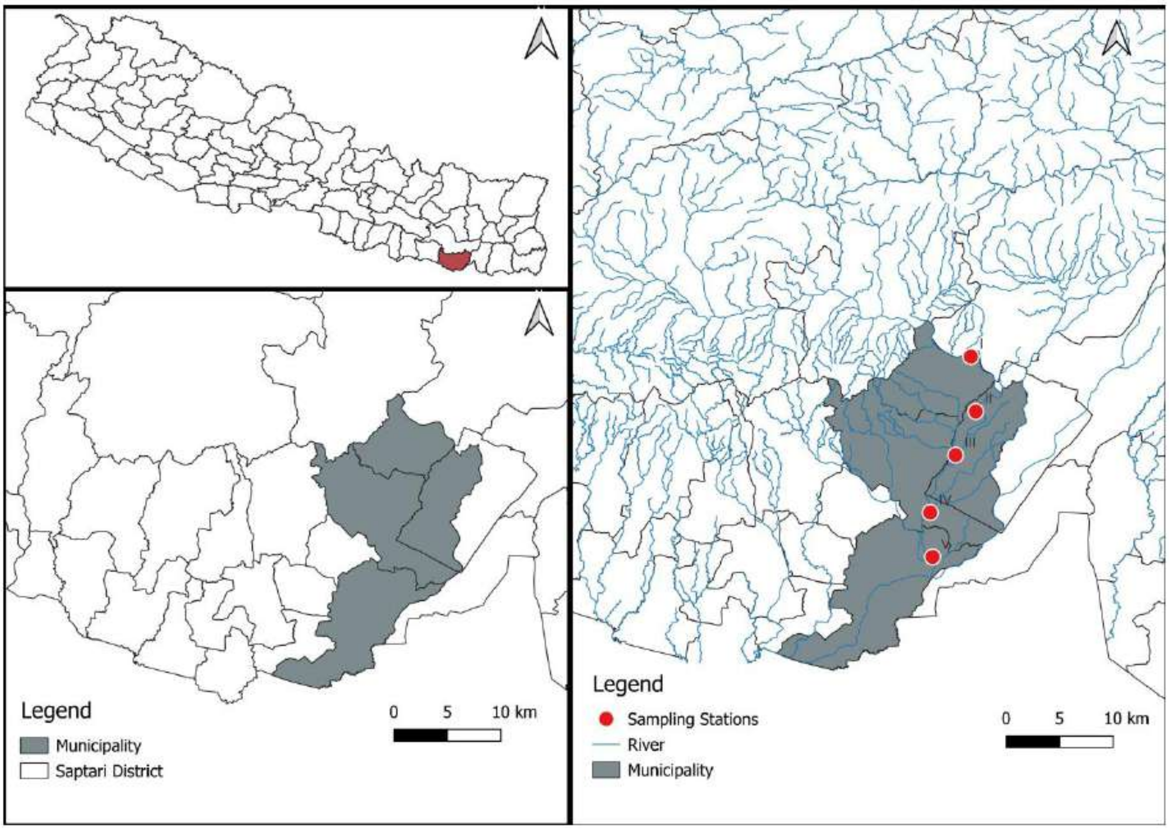

Figure 1. Map of the study area

3.2 Sampling stations

Altogether five sampling stations were selected along the river sides covering 25 km distance. The stations were selected on the basis of anthropogenically distributed area, crowded area and undistributed area.The five sampling stations are listed as below:

Site I

The sampling station I (26° 43' 58.38"N, 86° 57' 26.65"E, elevation: 123m) was chosen at Phatteypur which is about 3 km away Phatteypur bazar. The river becomes flat with medium water velocity and water depth of 108.92 cm at this station, and the river bottom contains a large amount of sands pebbles. There is no agricultural land. There is human settlement near the station.

Site II

The sampling station II (26° 40' 53.18"N, 86° 57' 59.05"E, elevation: 93m) was chosen at Kamalpur which is about 1.5 km away from odraha chowk and 5 km downstream from the station I. The river becomes narrow with low water velocity and depth of 112.22 cm at this station, and the river bottom contains a large amount of sands. There is also agricultural land on the other side. There is no human settlement in the vicinity of the station.

Site III

The sampling station III (26° 38' 25.80"N, 86° 56' 51.09"E, elevation: 86m) was chosen at Pathari, which is approximately 9 km north of the Mahendra Highway in Vardaha and 4km downstream from kamalpur. A large amount of pebble gravel and sand were discovered in the boulders at this station. The current state of the river bed was due to the water being clear and cold, with a medium velocity of water compared to other stations. On the river's bank, there was human settlement. Locals used to steal construction materials such as sand, boulders, and pebbles, as well as engage in illegal fishing. Despite this, more fish were discovered in this study area.

Site IV

The sampling station IV (26° 35' 12.07"N, 86° 55' 25.70"E, elevation: 82m) was at Bairwa, near Barmajhiya chowk which is 6 km downstream from station III. A large amount of pebble gravel and sand were discovered at this station. The current state of the river bed was due to the water being clear and cold, with a medium velocity and depth of 92.3 cm of water compared to other stations. The people were washing their clothes and performing seasonal tasks. On the river's bank, there was human settlement.

Site V

The sampling station V (26° 32' 42.72"N, 86° 57' 33.76"E, elevation: 77m) was chosen at Koshi baan, which is 5km downstream of station IV. The river becomes flat with high water velocity at this station and depth of 97.3 cm, and the river bottom contains a large amount of sands. There is also agricultural land on the other side. There is no human settlement in the vicinity of the station.

3.3 Sampling time period

The fieldwork was carried out three times between June to December 2024, during the months of June (summer), September (autumn), and December (winter).

3.4 Fish sampling, preservation techniques and identification

In this study, fish were collected from different stations with the help of local fishermen using locally available equipment like cast nets. The samples were collected using cast nets with a mesh size of 1cm*1cm during the morning hours between 7:00 and 10:00 AM, across summer, autumn, and winter. Sampling was conducted within 3 km i.e. 1.5 km upstream and 1.5 km downstream of each station, with 15 throws of the cast net at each station.

Fish specimens were photographed on-site using an Oppo A57 mobile phone. Identification was carried out both in the field and at the CDZ laboratory using the identification guides by, Jayaram (2010) and Shrestha (2019). Identified fish were immediately released back into the river following standard ethical and scientific procedures to minimize stress and physical harm. Collected samples were kept in a hundi filled with river water to maintain optimal conditions and reduce stress before release. During handling, efforts were made to keep the fish in water whenever possible. Fish were handled gently, avoiding contact with sensitive areas such as the gills and eyes, and were supported horizontally to prevent injury. When necessary, fish were revived by holding them in an upright position facing upstream, allowing water to flow through their gills until normal swimming behavior resumed. Once the fish regained strength, they were released back into suitable river conditions, ensuring their safe return to their natural habitat and unidentified fish were preserved in 10% formalin for further study at the CDZ lab. The water sample, preserved in an ice-filled container, was transported to the CDZ laboratory and analyzed within 24 hours of collection.

3.5 Analysis of physico-chemical parameter of water

3.5.1 Physical parameter of water

The physical parameters that were analyzed in each stations include;

I. Water temperature

Digital thermometer was used to measure the water temperature (℃).

II. Surface water velocity

Surface water velocity was measured using a straightforward method by timing a plastic bottle tied to a rope as it floated down the river. A stopwatch recorded the time taken to travel a specific distance, and the velocity was calculated in meters per second (m/s).

Formula to calculate surface water velocity:

Velocity =Distance(d)/ Time taken(t)

Where,

d = distance traveled by the float
t = time taken to travel that distance

III. Transparency

Water transparency was measured using a Secchi disc. The disc was lowered into the water, and the depth in centimeters (cm) was recorded using a measuring tape. It was then slowly lifted, and the depth at which it became visible again was noted.

Formula to calculate transparency:

Transparency = (Depth of disappearance + Depth of reappearance)/ 2

3.5.2 Chemical parameters of water

The chemical parameters that were measured include:

I. Hydrogen ion concentration (pH)

The concentration of hydrogen ions in water was measured by pH meter (pHep®, HI 98106), which was a measure of the intensity of acidity and alkalinity.

II. Dissolved Oxygen (DO)

A water sample was carefully filled into a DO bottle to measure the dissolved oxygen, making sure no air bubbles got inside. After adding two millilitres of manganese sulphate and two millilitres of alkali-iodide-azide, the bottle was promptly sealed with a stopper. Two millilitres of strong sulphuric acid were added to the sealed bottle after four to five minutes of waiting. A measuring cylinder was then put in the DO bottle after being immersed in sodium thiosulphate solution (0.025N). The colour of the fluid shifted from yellow to colourless. The following formula provides the computation for figuring out the DO in the water sample:

$DO (mg/l) = [(V_1-V_2)*N*8000]/V$

Where,

V= Volume of water sample
V_1 = Volume of titrant used for the sample
V_2 = Volume of titrant used for blank
N = Normality of the sodium thiosulphate solution

III. Free Carbon Dioxide (CO_2)

Two to three drops of phenolphthalein indicator were applied to 100 millilitres of water in a conical flask in order to determine the amount of free carbon dioxide in the sample. Free carbon dioxide was present because the solution stayed colourless. The titration process was then carried out using a standard alkali solution (0.05N NaOH). When the solution became light pink, the endpoint was recorded. The following formula provides the computation for figuring out the amount of free CO2 in a water sample:

Free CO_2 (mg/l) = (ml*N of NaOH*1000*44)/V

Where,

V=Volume of water sample

IV. Alkalinity:

A conical flask containing 50 cc of water was used to measure the alkalinity of the sample. The absence of carbonate or hydroxide alkalinity was demonstrated by the fact that no colour change happened when a few drops of phenolphthalein were introduced. The solution was then made yellow by adding a few drops of methyl orange indicator. After that, 0.1 N HCl was added to the solution until it turned pink. The total amount of HCl used at each sampling location was noted in order to calculate the alkalinity level of the water.

The following formula can be used to determine alkalinity:

Alkalinity as $CaCO_3$(mg/l) = (A*N of HCl*1000*50)/V

Where,

A= volume of HCl consumed during titration

V= volume of water sample

V. Hardness:

The burette was filled with a standard EDTA solution upto the zero level. A 50 ml sample of water was taken in a flask and 1 ml of ammonia buffer was added to the solution. Subsequently, 5-6 drops of Erichrome black-T indicator were introduced, resulting in the solution turning into a wine-red colour. The initial reading was then recorded. The solution was titrated against the EDTA solution until the wine-red colour turned into a blue hue, and the final reading was noted. This process was repeated until three concurrent values were obtained.

The following formula can be used to determine hardness:

Total hardness of water as $CaCO_3$ (mg/L) = (ml of EDTA used*1000)/V

Where,

V= volume of water sample

VI. Total dissolved solids (TDS):

All inorganic and organic substances that are floating in a liquid, whether they are molecules, ions, or micro-granular (colloidal sol), are measured by the total dissolved solids (TDS). Parts per million (ppm) is a typical unit of measurement for TDS. A digital TDS meter was used to measure the amount of TDS in water.

VII. Electrical conductivity (EC):

The ability of water to carry an electric current is known as electrical conductivity. It is a measurement of the electric flow transmission capacity of water. It also gives details about the water's quality and composition. The conductivity of water is affected by the presence of dissolved ions, or charged particles. Salts, acids, and bases are only a few of the dissolved substances that can produce these ions. Siemens per meter (S/m) or microsiemens per centimetre (μS/cm) are common units of measurement for conductivity. A digital EC meter was used to measure the electrical conductivity.

Species diversity index:

The diversity of species was calculated by using Shannon-Weiner diversity index (Shannon and Weiner, 1949).

Shannon Weiner diversity is denoted as H' which is calculated as,

$H' = \sum(P_i)*\ln(P_i)$

Where,

Pi = n/N

N = number of all individual of a species

N = Total number of all individuals in the sample

Ln = Logarithm of base e

Species richness index:

The species richness is calculated by using Margalef species richness (Margalef, 1968), which is denoted by d and calculated as,

Species richness(d) = S-l/logN

Where,

S = Total number of species

N = Total number of individuals in the sample

Eveness Index:

The evenness index was calculated using the following equation (Pielou, 1974).

Evenness (J) = H'/log S

Where,

H '= Shannon – Weiner index

S = Total number of species in the sample

3.6 Statistical analysis

For the statistical analysis, R software was used where the calculation were formed to determine the Shannon-Weiner index, species richness, evenness index for various seasons and sites. To analyse the association between species abundance, season, sites and environmental variables, redundancy analysis (RDA) was employed. This method is based on a linear response of species to environmental variables was chosen over canonical correspondence analysis (CCA) after considering the results of Detrended correspondence analysis (DCA). The axis length (< 3.5) and eigen value (< 50%) obtained from DCA indicated that the linear model associated with RDA was more suitable for the data.

4. Results

4.1 Ichthyofaunal diversity and distribution

4.1.1 Systemic position of the fishes

The present study identified 32 fish species (Photo Plate: I-XII), which were further classified into 7 orders, 15 families and 25 genera (Table 1).

Table 1. Systemic position of fishes

Order	Family	Scientific name of Species	Local name	Station
Cypriniformes	Danionidae	*Barilius barila* (Hamilton, 1822)	Koshiya	I, II, III, IV, V
		Barilius vagra (Hamilton, 1822)	Koshiya	I, II, III, IV, V
		Opsarius shacra (Hamilton, 1822)	Koshiya	III
		Cabdio morar (Hamilton, 1822)	Maror	I, II, III, IV, V
		Chela cachius (Hamilton, 1822)	Chana	I, II, III
		Chagunius chagunio (Hamilton, 1822)	Rewa	I, III
		Raiamas bola (Hamilton, 1822)	Goha	III
		Raiamas guttatus (Day, 1870)	Goha	I, II, III, IV, V
		Rasbora daniconius (Hamilton, 1822)	Deduwa	II
		Salmostoma acinaces (Valenciennes, 1844)	Chelha	I, II, III, V
	Cyprinidae	*Garra annandalei* (Hora, 1921)	Buduna	I, II, III, IV
		Puntius sophore	Pothi	I, II, III, IV, V

		(Hamilton, 1822)		
		Pethia ticto (Hamilton, 1822)	Pothi	I, II, III, IV, V
		Systomus sarana (Hamilton, 1822)	Bada Pothi	I, III, V
	Nemacheilidae	*Acanthocobitis botia* (Hamilton, 1822)	Latta	I, II, III, IV, V
Siluriformes	Bagridae	*Sperata aor* (Hamilton, 1822)	Kanti	I, V
		Mystus bleekeri (Day, 1877)	Tengra	I, II, III, IV, V
		Mystus cavasius (Hamilton, 1822)	Tengra	I, II, III, IV, V
	Sisoridae	*Gogangra viridescens* (Hamilton, 1822)	Padna	I, II, III, IV, V
	Heteropneustidae	*Heteropneustes fossilis* (Bloch, 1974)	Singhi	I, II
	Schilbeidae	*Pachypterus atherinoides* (Bloch, 1794)	Potasi	I, II, III
	Siluridae	*Wallago attu* (Bloch and Schneider, 1801)	Buwaari	I, II, III, V
Anabantiformes	Channidae	*Channa punctata* (Bloch, 1793)	Garrai	I, II, III, IV, V
		Channa orientalis (Bloch and Schneider, 1801)	Chenga	I, II, III, IV, V
	Anabantidae	*Anabas cobajuis* (Hamilton, 1822)	Kotri	III
	Osphronidae	*Trichogaster lalius* (Hamilton, 1822)	Kotri	I, III, V
		Trichogaster fasciatus (Bloch and Schneider, 1801)	Kotri	I, II, III, IV, V

Synbranchiformes	Mastacembelidae	*Macrognathus aral* (Bloch and Schneider, 1801)	Gainchi	I, II, III, IV
		Mastacembalus armatus (Lacepede, 1800)	Baami	I, II, III, IV, V
Gobiiformes	Gobiidae	*Glossogobius giuris* (Hamilton, 1822)	Bulla	II
Mugiliformes	Ambassidae	*Parambassis baculis* (Hamilton, 1822)	Chunna	I, II
Beloniformes	Belonidae	*Xenentodon cancila* (Hamilton, 1822)	Kauwa Maachh	I

4.1.1.1 Order wise ichthyofaunal distribution

With 47% of the entire fish population in the Triyuga River belonging to the Cypriniformes order, the order with the largest distribution was followed by the Siluriformes, which comprised 22%. Anabantiformes, Synbranchiformes, Beloniformes, Gobiiformes and Mugiliformes comprised for 16%, 6%, 3%, 3% and 3%, respectively of the fish population (Fig.2).

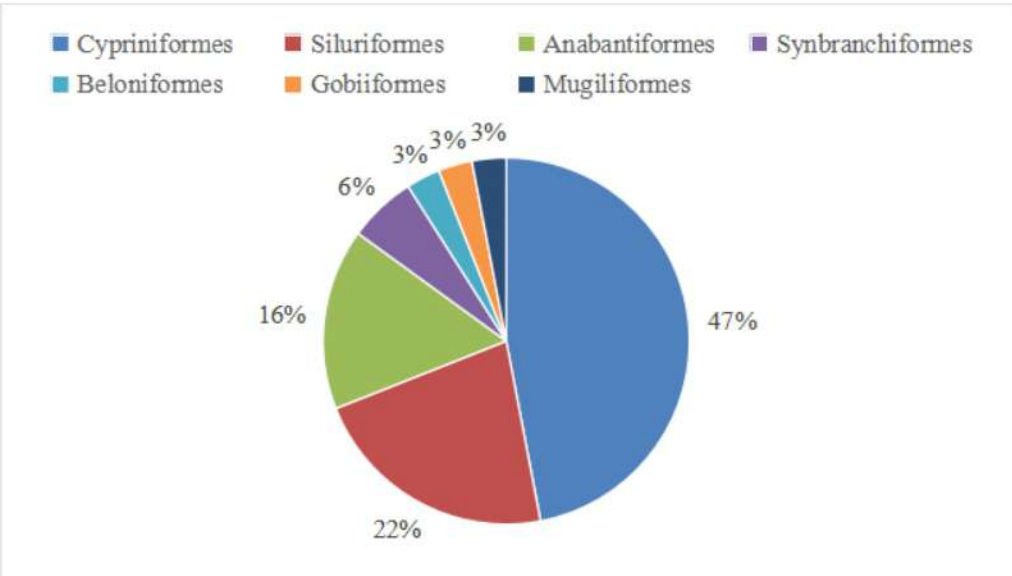

Figure 2. Order wise fish composition

4.1.1.2 Family wise ichthyofaunal distribution

The most prevalent fish family in the Triyuga River was Danionidae, accounting for 29% of the total fish population. The least abundant families were Nemacheilidae, Sisoridae, Heteropneustidae, Schilbeidae, Siluridae, Gobiidae, Ambassidae, Osphronemidae, and Belonidae, each of which accounted for only 3% of the total fish population (Fig.3).

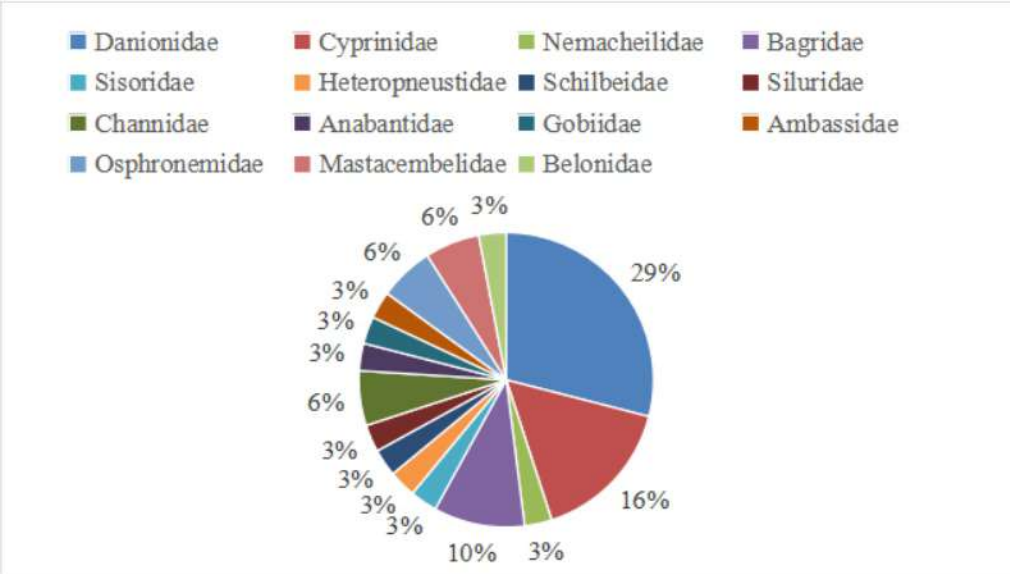

Figure 3. Family wise fish composition

4.1.1.3 Distribution pattern and frequency occurrence of fishes

Pachypterus atherinoides was the most prevalent fish species found in the Triyuga River, Saptari, accounting for 22.74% of the total fish population. The species with the lowest occurrence frequency were *Anabas cobajuis, Opsarius shacra, Rasbora daniconius, Raiamas bola*, and *Xenentodon cancila,* which constituted only 0.07% of the total fish population (Table 2).

Table 2. Distribution pattern and frequency occurrence of fishes

S.N.	Name of species	Local name	Season															Total	Frequency (%)
			Summer					Autumn					Winter						
			I	II	III	IV	V	I	II	III	IV	V	I	II	III	IV	V		
1.	*Acanthocobitis botia*	Latta	6	8	8	12	4	12	9	6	6	14	10	22	9	15	13	154	11.37
2.	*Anabas cobajuis*	Kotri	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0.07
3.	*Barilus barila*	Koshiya	2	2	1	1	5	0	0	1	3	2	5	1	2	0	3	28	2.06
4	*Barilius vagra*	Koshiya	0	0	2	0	3	1	2	1	1	4	3	0	2	1	3	23	1.69
5	*Cabdio morar*	Maror	9	11	10	4	4	5	9	0	3	14	18	11	6	0	3	107	7.90
6	*Chela cachius*	Chana	2	1	0	0	0	0	3	2	0	0	0	0	0	0	0	8	0.59
7	*Chagunius chagunio*	Rewa	1	0	2	0	0	0	0	1	0	0	0	0	0	0	0	4	0.29
8	*Channa punctata*	Garrai	2	1	0	0	0	3	4	1	0	3	4	0	0	4	0	22	1.62
9	*Channa orientalis*	Chenga	0	0	0	0	0	3	5	9	4	1	9	8	10	2	0	51	3.76
10	*Garra annandalei*	Buduna	3	1	2	0	0	0	0	2	0	1	2	2	1	0	0	14	1.03
11	*Glossogobius giuris*	Bulla	0	0	0	0	0	0	2	0	0	0	0	1	0	0	0	3	0.22
12	*Gogangra viridescens*	Padna	3	2	6	0	1	6	9	12	7	0	0	0	14	2	0	62	4.57
13	*Heteropneustes fossilis*	Singhi	0	0	0	0	0	0	0	0	0	0	3	0	6	0	0	9	0.66
14	*Macrognathus aral*	Gainchi	0	0	0	0	0	3	0	6	6	0	9	10	7	2	0	43	3.17
15	*Mastacembalus armatus*	Baami	2	1	3	0	0	0	0	2	1	3	0	0	4	1	0	17	1.25
16	*Mystus bleekeri*	Tengra	5	2	0	6	0	6	0	4	2	2	0	7	4	0	0	38	2.80
17	*Mystus cavasius*	Tengra	3	5	10	4	11	10	7	9	0	3	5	9	0	4	0	80	5.90
18	*Opsarius shacra*	Koshiya	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0.07
19	*Pachypterus*	Potasi	50	29	51	0	0	27	32	50	0	0	33	14	22	0	0	308	22.74

	atherinoides																	
20	*Parambassis baculis*	Chunna	0	1	0	0	0	2	0	0	0	0	0	0	0	0	0	3	0.22
21	*Pethia ticto*	Pothi	4	6	9	0	0	0	4	0	7	0	5	7	10	9	5	66	4.87
22	*Puntius sophore*	Pothi	11	9	8	12	19	7	5	6	8	13	20	24	24	8	2	176	12.99
23	*Raiamas bola*	Goha	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0.07
24	*Raiamas guttatus*	Goha	2	1	6	2	9	1	0	3	0	1	1	2	6	0	4	38	2.80
25	*Rasbora daniconius*	Deduwa	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0.07
26	*Salmostoma acinaces*	Chelha	4	3	2	0	6	3	2	7	0	9	0	1	5	0	0	42	3.10
27	*Sperata aor*	Kanti	0	0	0	0	0	1	0	0	0	2	0	0	0	0	0	3	0.22
28	*Systomus sarana*	Bada pothi	1	0	2	0	1	0	0	0	0	0	0	0	0	0	0	4	0.29
29	*Trichogaster lalius*	Kotri	0	0	0	0	0	1	0	2	0	0	0	0	0	0	02	5	0.36
30	*Trichogaster fasciatus*	Kotri	2	1	0	0	3	5	3	6	1	0	0	0	4	0	0	25	1.84
31	*Wallago attu*	Buwaari	1	1	3	0	4	0	0	1	0	2	0	3	1	0	0	16	1.18
32	*Xenentodon cancila*	Kauwa maach	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0.07

4.1.2 Diversity status of ichthyofaunal

4.1.2.1 Spatial variation in fish assemblage

Station IV had the lowest species diversity (2.3), whereas station III had the highest value (2.6) according to the Shannon-Weiner diversity index (H'). The species richness index (d) of Margalef also revealed that Station IV had the lowest value (2.84) and Station I had the greatest (4.46). Additionally, station IV displayed the highest evenness value of 0.85 according to Pielou's evenness index, whereas station I displayed the lowest evenness value of 0.73 (Fig.4).

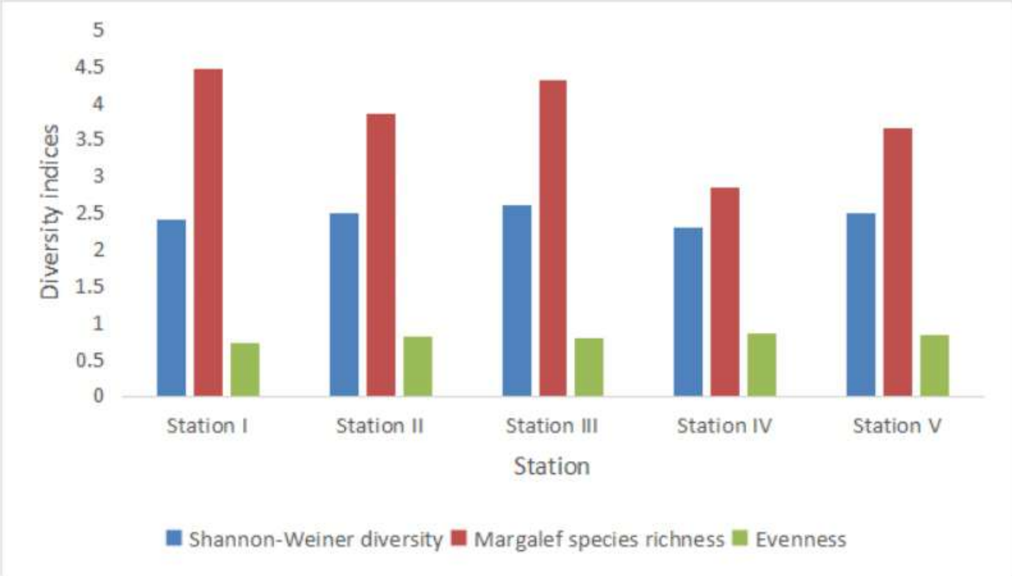

Figure 4. Spatial variation in fish assemblage

4.1.2.2 Temporal variation in fish assemblage

The Shannon-Weiner diversity index (H') indicated that Autumn had the highest level of species diversity (2.66), and Summer had the lowest, with a value (2.46). Similarly, Margalef's species richness index (d) showed that autumn had the greatest value of 4.08, followed by summer (3.94) and winter (3.41). Pielou's evenness index also showed that Winter has the highest evenness value (0.84), followed by Autumn (0.81) and Summer (0.76) respectively (Fig.5).

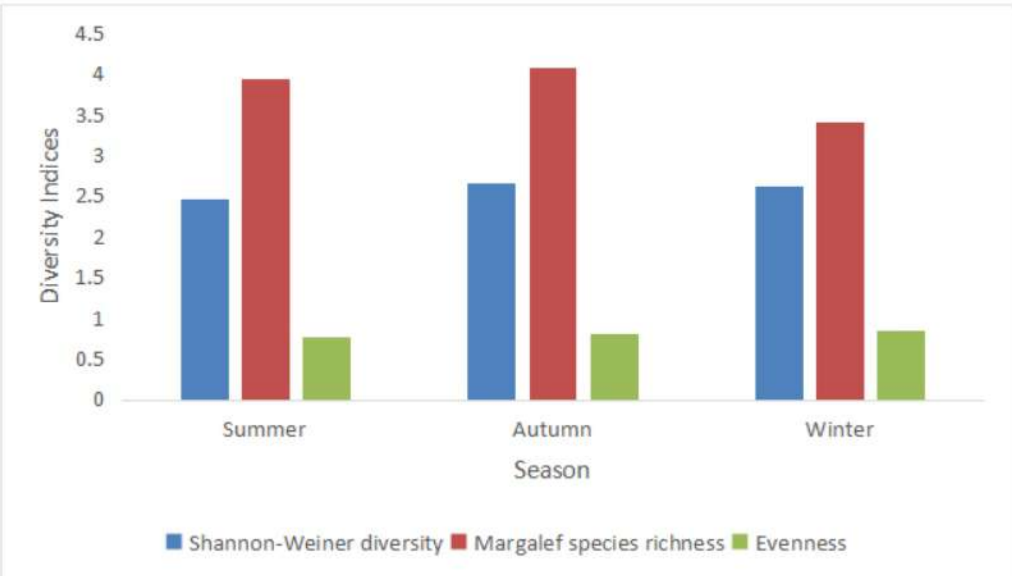

Figure 5. Temporal variation in fish assemblage

4.2 Variation in water quality parameters

4.2.1 Physical parameters of water

4.2.1.1 Water temperature

During the summer, station I recorded the highest water temperature of 33°C, while station IV had the lowest temperature of 17.9°C in winter. Stations I, II, III, IV and V had average temperature of 26°C, 25.4°C, 26.2°C, 25.8°C, and 26.3°C throughout the seasons. The average temperature in summer, autumn, and winter were 35.52°C, 26.68°C and 18.68°C. Overall, there was no significant difference in average temperatures among stations (Fig.6).

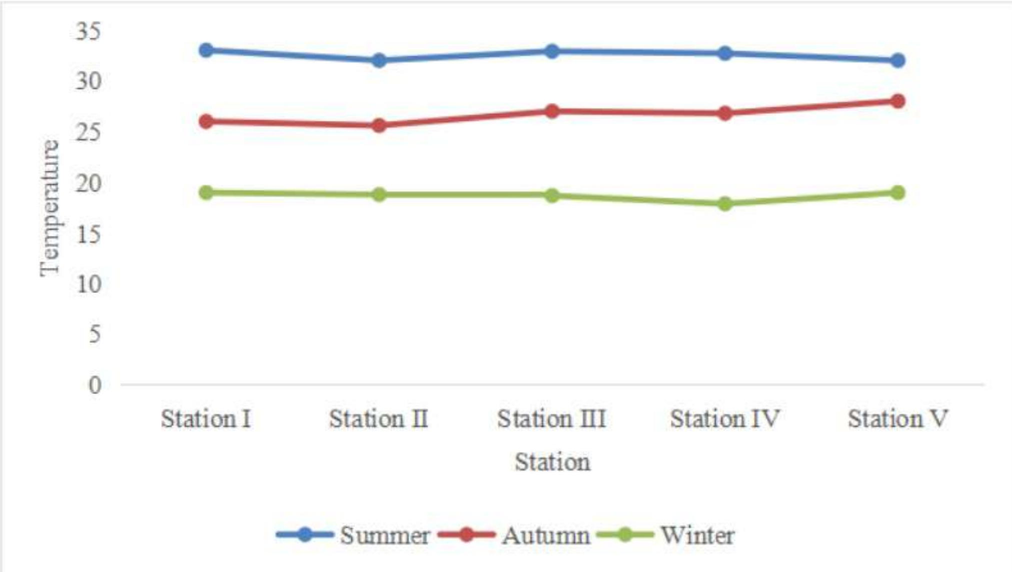

Figure 6. Variation in water temperature at different station and season

4.2.1.2 Surface water velocity

During the autumn, station V recorded the highest water velocity of 1.27 m/s, while station I had the lowest velocity of 1.10 m/s in winter. Stations I, II, III, IV and V had average velocities of 1.14 m/s, 1.16 m/s, 1.17 m/s, 1.15 m/s and 1.21 m/s throughout the seasons. The average velocities in summer, autumn, and winter were 1.18 m/s, 1.19 m/s and 1.14 m/s (Fig.7).

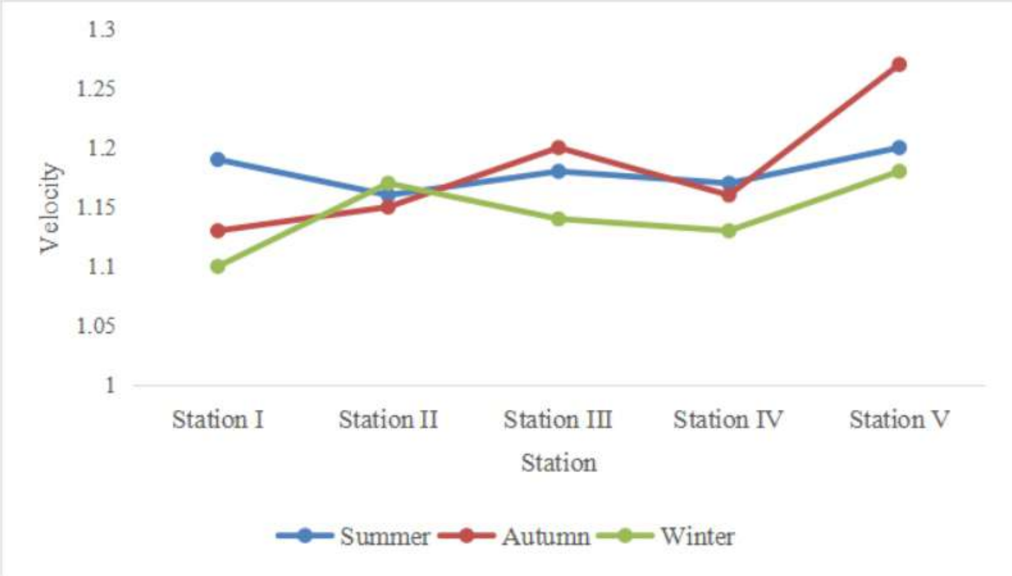

Figure 7: Variation in surface water velocity at different station and season

4.2.1.3 Water transparency

In the summer, station I had a highest water transparency of 89 cm, while station V had the lowest transparency of 37 cm in the winter. Stations I, II, III, IV, and V exhibited average transparency values of 88.17 cm, 66.5 cm, 55 cm, 85.84 cm, and 38.67 cm, respectively, throughout the season. The average transparency in summer, autumn, and winter was 67.3 cm, 66.9 cm, and 66.3 cm, respectively (Fig.8).

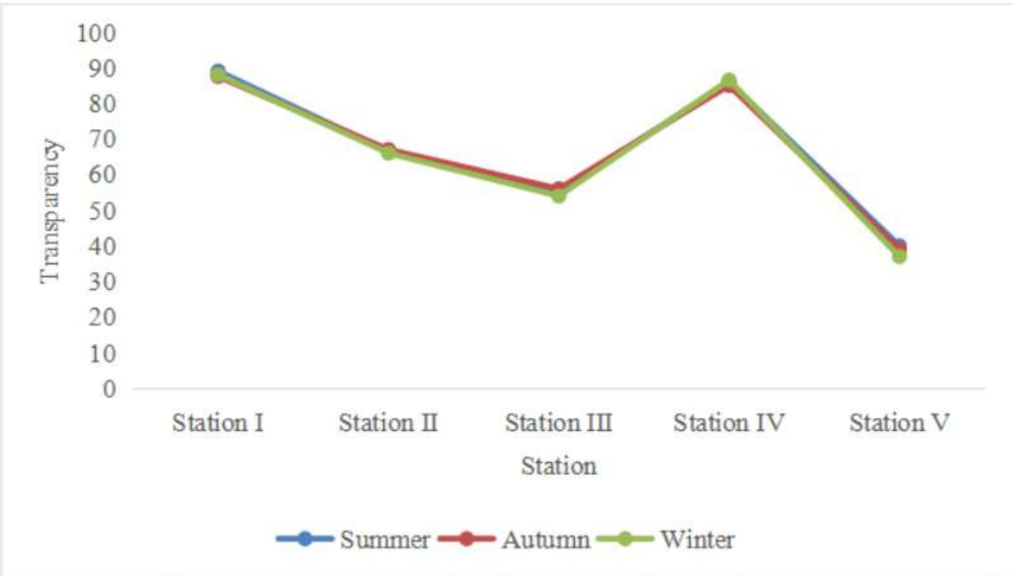

Figure 8. Variation in water transparency at different station and season

4.2.2 Chemical parameters of water

4.2.2.1 Hydroen ion concentration (pH)

In the summer, station II recorded a highest pH value of 8.5, while station V had the lowest pH value of 7.7 in the winter. Stations I, II, III, IV, and V exhibited average pH values of 8.2, 8.3, 8.1, 7.93 and 7.96 respectively, throughout the season. The average pH values in summer, autumn, and winter were 8.14, 8.24 and 8, respectively (Fig.9).

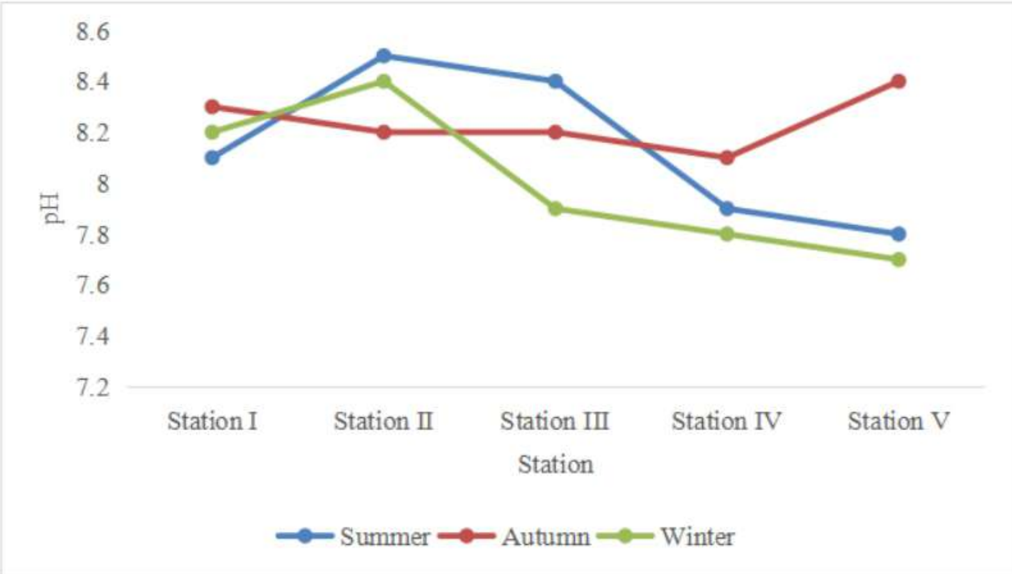

Figure 9. Variation in pH at different station and season

4.2.2.2 Free carbon dioxide (CO_2)

During the winter, station I recorded the highest free CO_2 concentration of 3.96 mg/L, while station V had the lowest free CO_2 concentration of 2.18 mg/L in summer. Stations I, II, III, IV and V had average free CO_2 concentration of 3.20 mg/L, 2.97 mg/L, 2.60 mg/L, 2.92 mg/L and 2.90 mg/L throughout the seasons. The average free CO_2 concentration in summer, autumn, and winter were 2.78 mg/L, 2.68 mg/L and 3.32 mg/L respectively (Fig.10).

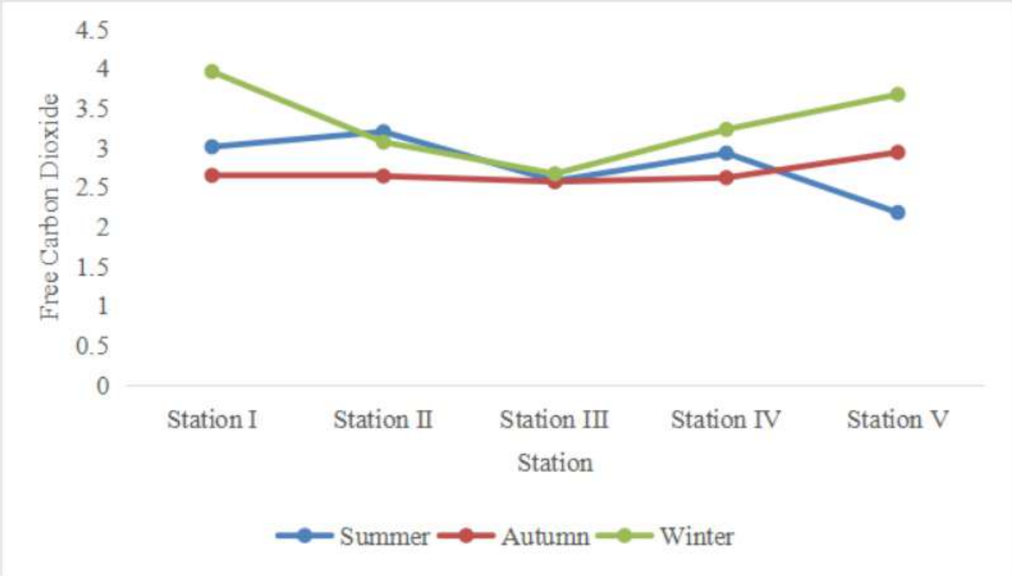

Figure 10. Variation in CO_2 at different station and season

4.2.2.3 Dissolved oxygen (DO)

In the winter, station IV recorded the highest dissolved oxygen level 7.25 mg/L, while station I had the lowest dissolved oxygen level of 5.67 mg/L in summer. Stations I, II, III, IV and V had average dissolved oxygen level of 6.07 mg/L, 6.89 mg/L, 6.56 mg/L, 6.55 mg/L and 6.42 mg/L throughout the seasons. The average dissolved oxygen level in summer, autumn, and winter were 5.95 mg/L, 6.53 mg/L and 7.02 mg/L respectively (Fig.11).

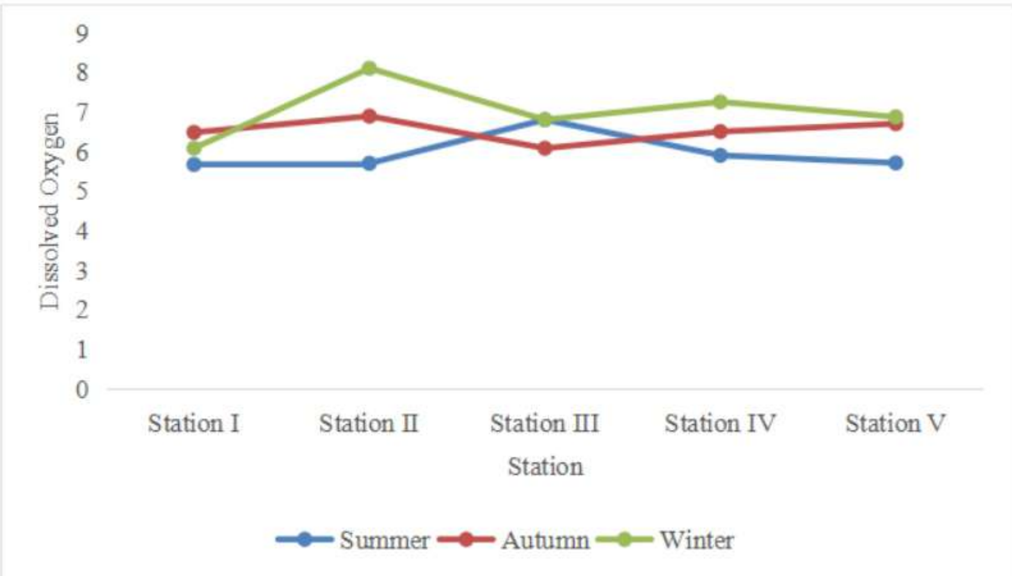

Figure 11. Variation in DO at different station and season

4.2.2.4 Total alkalinity (mg/L)

During the winter, station I recorded the highest alkalinity level at 39 mg/L, while station III had the lowest alkalinity of 25 mg/L in summer. Stations I, II, III, IV and V had average alkalinity level 33 mg/L, 32.67 mg/L, 28.33 mg/L, 31.33 mg/L and 34.75 mg/L throughout the seasons. The average alkalinity in summer, autumn, and winter were 30.4 mg/L, 29.71 mg/L and 35.93 mg/L respectively (Fig.12).

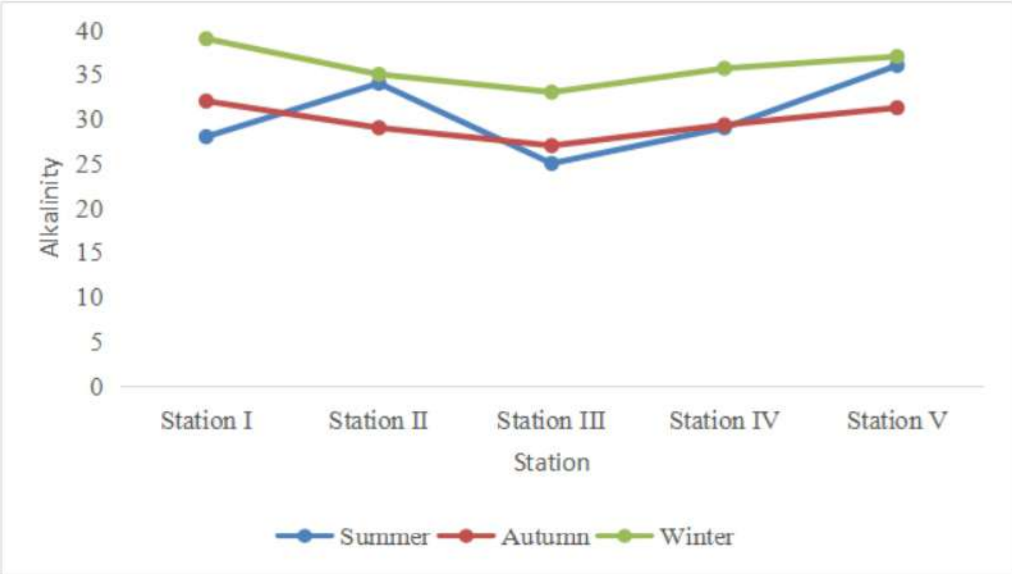

Figure 12. Variation in alkalinity at different station and season

4.2.2.5 Hardness (mg/L)

During the autumn, station I recorded the highest hardness value of 59.06 mg/L, while station II had the lowest hardness value of 47 mg/L in autumn. Stations I, II, III, IV and V had average hardness values of 55.68 mg/L, 53.1 mg/L, 53.35 mg/L, 56.47 mg/L and 56.17 mg/L throughout the seasons. The average hardness in summer, autumn, and winter were 56.18 mg/L, 55.68 mg/L and 53 mg/L respectively (Fig.13).

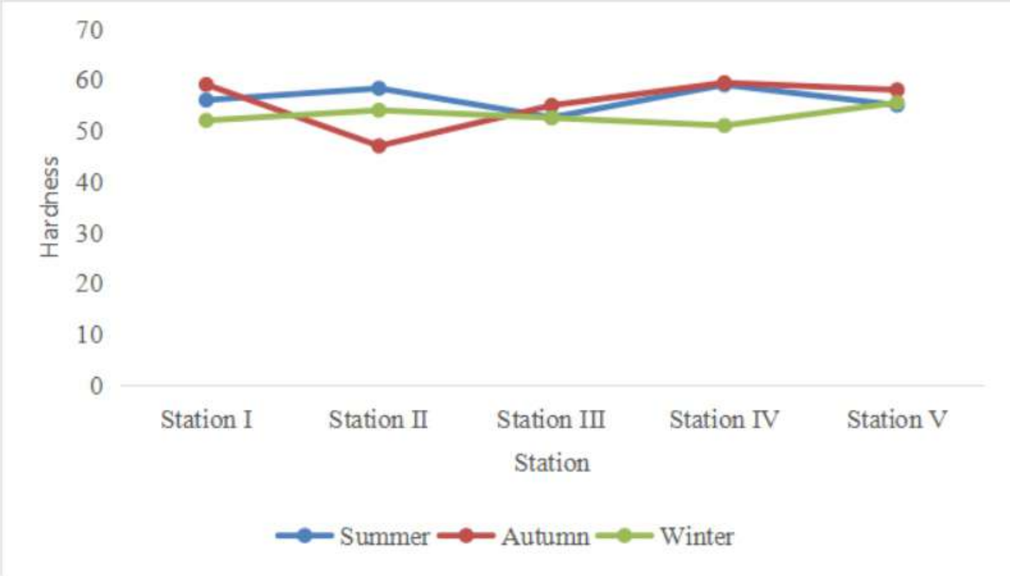

Figure 13. Variation in hardness at different station and season

4.2.2.6 Total dissolved solids (TDS)

In the winter, station IV recorded a highest TDS value of 170 ppm, while station I and IV had the lowest TDS value of 121 ppm in the winter. Stations I, II, III, IV, and V exhibited average TDS values of 140.34 ppm, 141.34 ppm, 144 ppm, 143.67 ppm and 139.67 ppm respectively, throughout the season. The average TDS values in summer, autumn, and winter were 123 ppm, 135.6 ppm and 166.8 ppm, respectively (Fig.14).

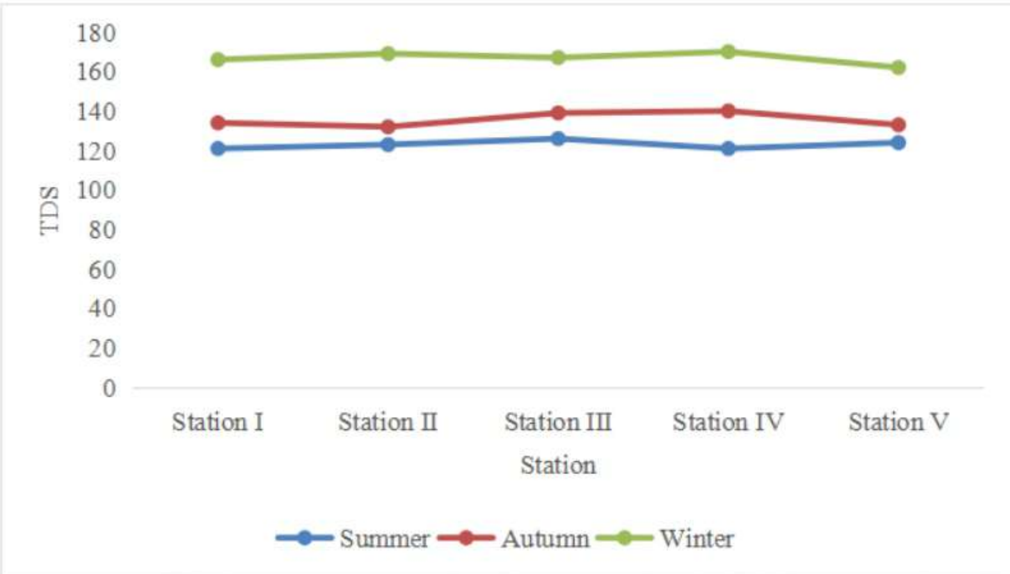

Figure 14. Variation in TDS at different station and season

4.2.2.7 Electrical conductivity (EC)

In the winter, station IV recorded a highest EC value of 340 µm/s, while station I and IV both had the lowest EC value of 241 µm/s in the winter. Stations I, II, III, IV, and V exhibited average EC values of 280.67 µm/s , 282.67 µm/s , 288 µm/s , 287.34 µm/s and 279.34 µm/s respectively, throughout the season. The average EC values in summer, autumn, and winter were 246 µm/s, 271.2 µm/s and 333.6 µm/s respectively (Fig.15).

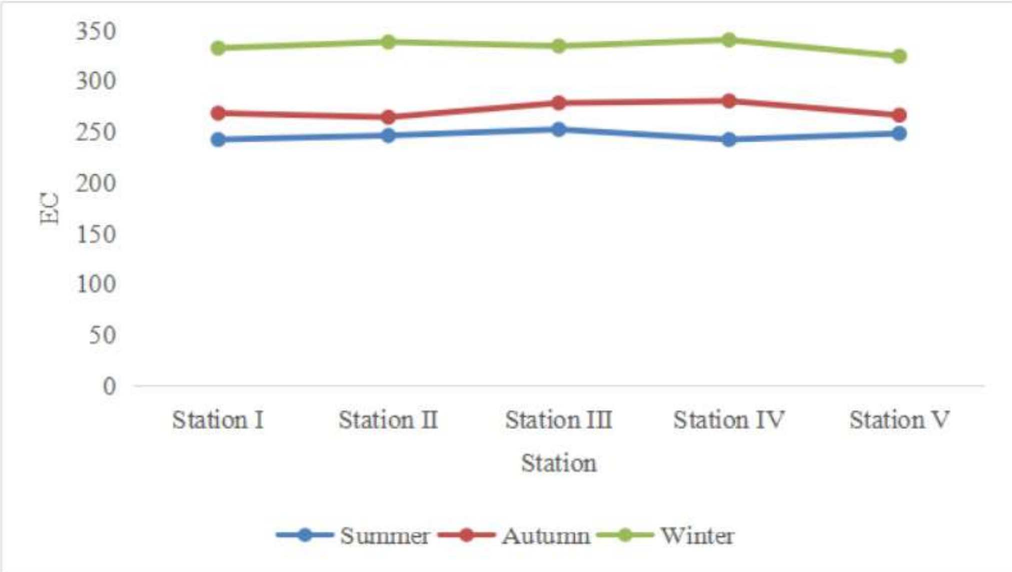

Figure 15. Variation in EC at different station and season

4.3 Relationship between fish species, seasonal and environmental variables

Standard Deviation (SD) units for the first and second Detrended Correspondence Analysis (DCA) axes were 1.67 and 1.29, respectively. The efficacy of Redundancy Correspondence Analysis (RDA) has been confirmed by the data matrix's overall variance (Table 3).

Table 3. DCA summary

	DCA1	DCA2	DCA3	DCA4
Eigenvalues	0.181	0.099	0.052	0.044
Decorana values	0.203	0.077	0.023	0.008
Axis lengths	1.674	1.296	0.715	0.836

The Redundancy Analysis (RDA) showed distinct interactions between different fish species and environmental variables.Temperature, pH, transparency, dissolved oxygen, free carbon dioxide, TDS, and alkalinity all showed significant relationships, with a strong negative association to velocity and hardness. After the DCA analysis, RDA plotting was performed using R software. Species were denoted as "S," and each species were coded as S1, S2, S3, ..., up to S32, respectively (Appendix 2).

In the RDA plot, occurrence of fish species such as *Chela cachius* (S6) and *Pachypteruss atherinoides* (S19) are highly associated with temperature. Specifically, *Cabdio morar* (S5), *Channa orientalis* (S9), *Gogangra viridescens* (S12), *Heteropneustes fossilis* (S13), *Macrognathus aral* (S14) and *Pethia ticto* (S21) exhibited positively significant relationships with transparency and pH. Similarly species like *Acanthocobitis botia* (S1) and *Puntius sophore* (S22) are highly associated with TDS, free carbondioxide, dissolved oxygen and alkalinity.None of the fish species were associated with velocity and hardness which exhibited negatively significant relationships (Fig.16).

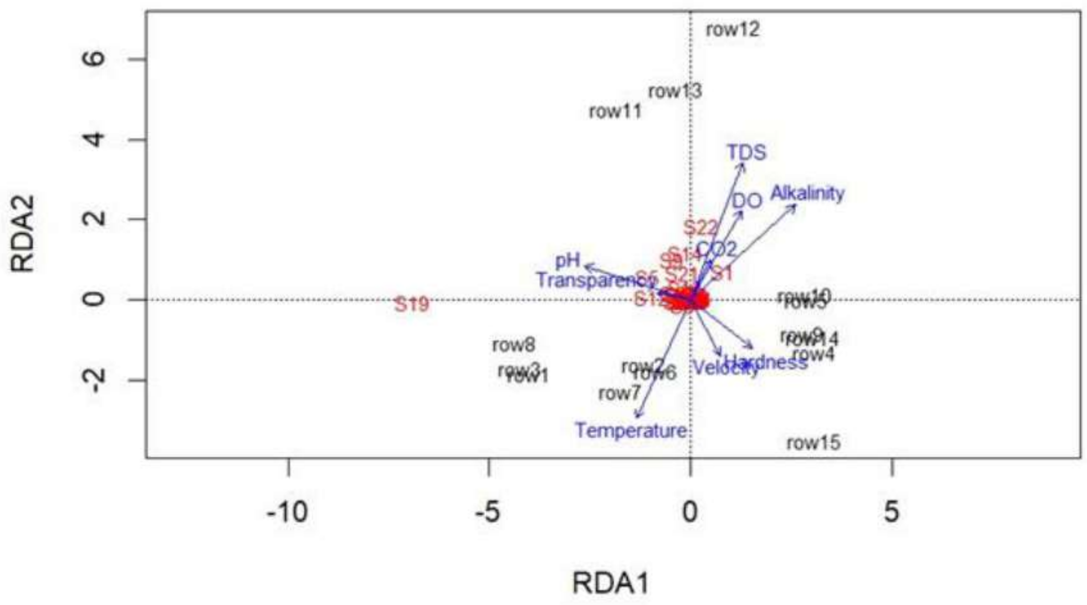

Figure 16. RDA analysis of species abundance with environmental variables

5. Discussion

5.1 Spatial variation in fish assemblage

The present study conducted in the Triyuga River, Eastern Nepal, documented a considerable diversity of ichthyofauna, identifying 32 species belonging to 7 orders, 15 families, and 25 genera. The predominance of *Pachypterus atherinoides* (22.74%), followed by *Puntius sophore* (12.99%) and *Acanthocobitis botia* (11.37%), suggests that these species are well-adapted to the prevailing environmental conditions of the river (Shrestha, 2016 and Shrestha, 2023). In contrast, species such as *Anabas cobajuis*, *Opsarius shacra*, *Rasbora daniconius*, *Raiamas bola*, and *Xenentodon cancila* were recorded in minimal numbers (0.07%), indicating either specific microhabitat requirements or limited distribution within the river system.

Furthermore, the dominance of the order Cypriniformes (47%) followed by Siluriformes (22%) observed in the current study supports findings from other river systems such as the Narayani, Babai, Ratuwa, and Tamor Rivers (Jha & Bhujel, 2014; Limbu & G.C., 2019; Limbu & Gupta, 2019; Tumbahangfe et al., 2021). These patterns align with broader national assessments, as noted in the Nepal Biodiversity Resource Book (Shakya et al., 2007), which emphasized the dominance of Cypriniformes in Nepal's freshwater ecosystems. Cyprinidae were predominant, suggesting that this riverine ecosystem share ecological similarities with Koshi River (Saud, 2011 and Shah, 2016), particularly in sustaining fish communities dependent on flowing and semi-stagnant freshwater systems.

Habitat preferences among fish species were evident during the study. Species such as *Macrognathus aral* and *Mastacembalus armatus* were predominantly associated with sandy and pebbly substrates coupled with moderate water flow, while species including *Heteropneustes fossilis*, *Channa punctata*, *Channa orientalis*, *Mystus bleekeri*, *Mystus cavasius*, and *Acanthocobitis botia* were frequently encountered in areas characterized by fine clay deposits and abundant submerged vegetation (Shrestha, 2016). The low abundance of several species, particularly *Anabas cobajuis* and *Xenentodon cancila*, may be attributed to limited habitat availability, microhabitat specialization, or ecological competition within the river system (Shakya et al. 2007) regarding the vulnerability of freshwater biodiversity to localized environmental changes and habitat fragmentation. The present study underscores the ecological richness of the Triyuga River and highlights the significance of habitat heterogeneity in supporting ichthyofaunal diversity. The findings contribute valuable baseline information necessary for the conservation and sustainable management of riverine fish diversity in Eastern Nepal, aligning with national biodiversity conservation priorities.

5.2 Temporal variation in fish assemblage

The current study on the fish diversity in the Triyuga River revealed substantial temporal variation in species richness, diversity, and evenness across the three seasons. Notably, *Pachypterus atherinoides, Puntius sophore,* and *Acanthocobitis botia* were consistently observed throughout all seasons, with *Pachypterus atherinoides* being the most dominant and abundant species, followed by *Puntius sophore*. This dominance contrasts with the findings of Shrestha (2016), who classified *Pachypterus atherinoides* as an occasionally occurring species in his study. The present study, however, indicates that *Pachypterus atherinoides* has adapted well to the prevailing environmental conditions in the Triyuga River, suggesting a temporal shift in its ecological dominance.

The Shannon-Weiner diversity index (H') was highest in the autumn (2.66) and lowest in the summer (2.46), indicating greater species diversity in the autumn. This is likely due to more favorable environmental conditions, including moderate temperature and stable flow rate of water, which support a higher variety of fish species. In contrast, the summer's lower diversity could be attributed to the elevated water temperatures and decreased oxygen levels, which create stress on aquatic life and reduce species variety. These findings are in line with Tamor River (Tumbahangfe et al., 2021), the lowest diversity index occurred in winter (2.56), and the highest was recorded during summer (3.01). These temporal changes reflect the adaptive responses of fish communities to varying environmental pressures throughout the year. Furthermore, station-wise differences in diversity were also observed in the Triyuga River, with Station III exhibiting the highest Shannon diversity index (2.6), while Station IV had the lowest (2.3). This variation underscores the importance of localized environmental factors, such as substrate composition, water depth, and flow rates, which influence fish distribution. These findings resonate with those from the Koshi River (Saud, 2011).

The Margalef's species richness index (d) further illustrated that autumn had the highest species richness (4.08), while winter recorded the lowest (3.41). This seasonal pattern suggests that the availability of favorable breeding conditions and habitat heterogeneity following the monsoon season promote greater species richness. Conversely, the lower species richness in winter may be due to several factors, including reduced metabolic activity of cold-blooded fish and the migration of species to deeper or warmer areas. This is consistent with observations from the Koshi River, where species richness is known to fluctuate significantly with seasonal changes, as fish species migrate in response to varying water levels and temperature shifts (Shah, 2016).

The Evenness index, which measures the relative abundance of species in a community, was highest in winter (0.84) and lowest in summer (0.76). The higher evenness in winter suggests that fish populations were more uniformly distributed across species, likely due to decreased competition for resources when fewer species are active. In contrast, the reduced evenness in summer could be attributed to the dominance of species that are better adapted to higher temperatures, such as *Puntius sophore* and *Acanthocobitis botia,* resulting in a less uniform distribution of species in the river. The dominance of a few species during the summer months mirrors findings from other rivers in Nepal, such as the Narayani River, where species like *Puntius sophore* and *Acanthocobitis botia* are more common during warmer months (Jha & Bhujel, 2014). During the monsoon season, when water levels rise and flow rates increase, both the Triyuga and Koshi rivers experience higher species richness and diversity due to enhanced habitat availability. Conversely, in the dry season, lower water levels and higher temperatures tend to stress fish populations, leading to decreased species diversity and evenness, as observed in the present study (Yadav, 2017).

The study also underscores the significance of environmental parameters, such as water temperature and dissolved oxygen levels, in shaping temporal variations in fish diversity. These factors play a critical role in the survival and distribution of species across different seasons, as noted in similar studies of Nepalese rivers (Limbu & Gupta, 2019; Tumbahangfe et al., 2021). The ability of species to adapt to these seasonal shifts is crucial for maintaining biodiversity, and ongoing monitoring is necessary to understand how climate change and anthropogenic activities may further affect the temporal dynamics of fish populations in these rivers. The Triyuga River's fish diversity exhibits significant temporal variation that mirrors seasonal shifts observed in other river systems in Nepal, such as the Koshi and Tamor rivers. The findings contribute valuable baseline information necessary for the conservation and sustainable management of riverine fish diversity in Eastern Nepal, aligning with national biodiversity conservation priorities Future research should focus on long-term environmental monitoring and sustainable river management.

5.3 Variation in fish assemblage structure with water quality

The present study provides valuable insights into the physicochemical parameters influencing the aquatic ecosystem of the Triyuga River and highlights their impact on fish diversity and health. Water temperature, dissolved oxygen (DO), pH, and other water quality parameters play critical roles in shaping the physical, chemical, and biological characteristics of freshwater ecosystems (Gulzar & Abubakr, 2019; Toufeek & Korium, 2009). The water temperature in the Triyuga River was highest in the summer (33°C) and lowest in winter (17.9°C). Temperature significantly affects biological activity, nutrient recycling, and gas solubility, all of which influence the distribution of aquatic organisms (Gulzar & Abubakr, 2019). In this study, the summer temperature peak (33°C) aligns with general seasonal patterns found in Nepalese rivers. For instance, Sharma and Shrestha (2001) reported a similar temperature range in the Tinau River, with summer temperatures reaching 32.5°C and winter temperatures as low as 16.5°C. These temperature variations can lead to seasonal stress for aquatic species, influencing their behavior, reproduction, and survival. Warmer temperatures in the summer generally lead to higher metabolic rates in fish, while cooler winter temperatures can result in decreased activity and metabolic functions, as shown by the temperature trends in both the Triyuga and Tinau rivers.

In the Triyuga River, DO levels varied from 5.67 mg/L in summer to 7.25 mg/L in winter. The higher DO in winter can be attributed to increased water transparency, which allows better light penetration and enhances photosynthetic oxygen production by aquatic plants (Limbu & Prasad, 2020). This finding is consistent with similar studies conducted in other rivers in Nepal. For example, Limbu and Prasad (2020) reported that the Nuwa River had DO values ranging from 5.5 mg/L to 7.0 mg/L. Higher DO levels in winter are beneficial for maintaining healthy fish populations, as oxygen-rich environments support sensitive species, including those that require high oxygen levels for survival, such as *Channa punctata* and *Acanthocobitis botia* in the Triyuga River. The pH of the water in the Triyuga River ranged from 7.7 to 8.5, with station averages of 7.93 to 8.3. These values are within the optimal pH range for most freshwater fish, which typically thrive in water with a pH between 6.5 and 9.0. Fluctuations outside this range can cause stress or mortality in fish populations, potentially altering community composition (Shah, 2016). This range is similar to findings from the Nuwa River, where the pH ranged from 7 to 9 (Limbu & Prasad, 2020). The Triyuga River's pH values suggest a stable environment for the species present. Furthermore, the river's alkalinity varied from 25 to 39 mg/L, which plays an essential role in buffering the water against pH changes. Low alkalinity rivers are more vulnerable to pH fluctuations, which can negatively affect fish populations. The Triyuga River's moderate alkalinity levels suggest a stable environment for freshwater fish species, supporting findings from other studies on Nepalese rivers (Shrestha, 2016). The water velocity in the Triyuga River was highest in autumn (1.27 m/s) and lowest in winter (1.10 m/s), likely due to seasonal rainfall and runoff fluctuations. This variation in water velocity influences habitat availability and the ability of species to migrate or access feeding grounds. Fish species that are adapted to higher velocities, such as *Channa orientalis* and *Macrognathus aral,* are better suited to areas with fast-moving water, while slower-moving species thrive in calmer sections of the river. The CO_2 concentration in the Triyuga River was found to be relatively low, ranging from 2.18 mg/L in summer to 3.96 mg/L in winter, which is consistent with findings from the Tamor River (Saund et al., 2010). CO_2 levels below 5 mg/L typically do not present a direct threat to fish, although excessive CO_2 concentrations can lead to reduced oxygen availability, stressing aquatic organisms. Water hardness in the Triyuga River varied from 47 to 59.06 mg/L, which indicates that the water is relatively soft, a feature that is generally favorable for freshwater fish species. Hardness is a measure of the dissolved calcium and magnesium ions in water, and higher hardness can affect fish osmo-regulatory processes. The TDS (Total Dissolved Solids) ranged from 121 ppm to 170 ppm, reflecting the amount of dissolved minerals in the water. These values suggest that the water in the Triyuga River is not overly mineralized, which is typical for many rivers in Nepal, as reported in studies on the Koshi River (Shah, 2016).

The Triyuga River's physicochemical parameters, including temperature, dissolved oxygen, pH, and water velocity, exhibit significant seasonal variation that impacts fish diversity and health. The findings of this study corroborate those of similar investigations in other river systems in Nepal, such as the Koshi River and Tamor River. The seasonal fluctuations in water temperature, oxygen levels, and pH can influence the distribution, behavior, and survival of fish species, highlighting the importance of maintaining stable water quality conditions for preserving aquatic biodiversity. Further research is needed to explore the long-term impacts of climate change and human activities on the water quality and fish populations of Nepalese rivers. Redundancy Analysis (RDA) plotting further revealed strong correlations between species distribution and environmental variables, emphasizing the impact of seasonal changes on fish assemblages. These results align with studies from other Nepalese rivers, such as the Koshi and Tamor Rivers, highlighting the importance of environmental factors in shaping aquatic ecosystems.

6. Conclusions and recommendations

6.1 Conclusions

This study assessed fish diversity in the Triyuga River, identified 32 species, which was influenced by seasonal variations in environmental parameters such as water temperature, dissolved oxygen, and pH significantly influenced fish diversity, with the highest diversity observed in autumn and the lowest in summer. The present study, however, indicates that *Pachypterus atherinoides* has adapted well to the prevailing environmental conditions in the Triyuga River, suggesting a temporal shift in its ecological dominance. The findings highlight the complex interplay between environmental factors and species distribution, emphasizing the need for conservation efforts that consider both seasonal and habitat-specific variations.

6.2 Recommendations

- The study highlights the effect of the seasonal variations, climate change, and human activities, therefore, regular assessments of fish diversity in the Triyuga River are crucial to monitor changes in species and ecosystem health, guiding conservation efforts and ensuring sustainable fish populations.
- Future studies by the researchers should continue to explore the mechanisms driving these temporal changes and their implications for the management of freshwater biodiversity in Nepal.
- The local government should form cooperative to provide technical and financial, support to local fishermen for promoting sustainable fishing practices. This support will help improve their livelihoods while contributing to the conservation of fish diversity and ensuring the long-term health of the river's ecosystem.

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Photo plate-I

Fish belonging to the order Cypriniformes

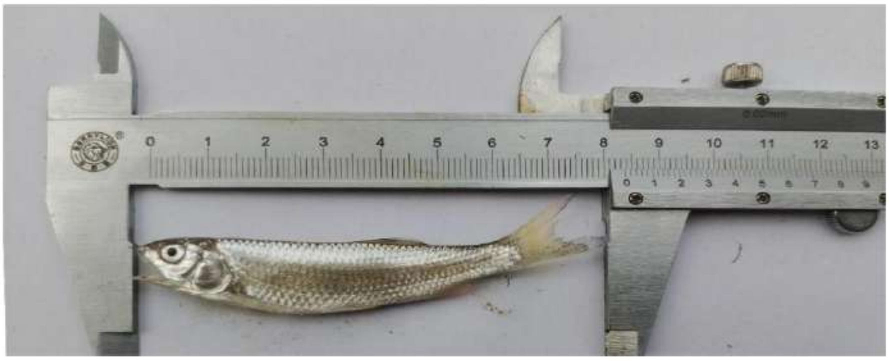

Photo 1: *Barilius barila*

Photo 2: *Barilius vagra*

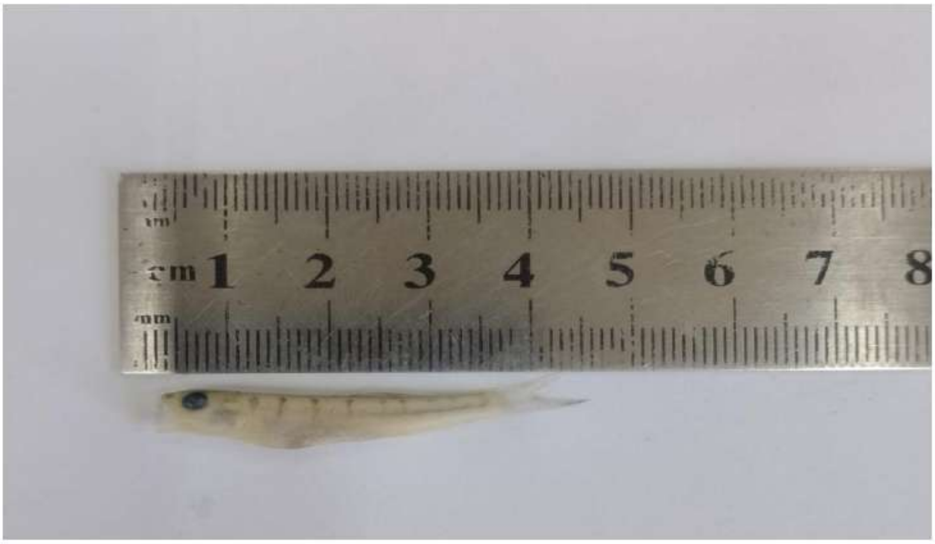

Photo 3: *Opsarius shacra*

Photo Plate-II

Fish belonging to the order Cypriniformes

Photo 4: *Cabdio morar*

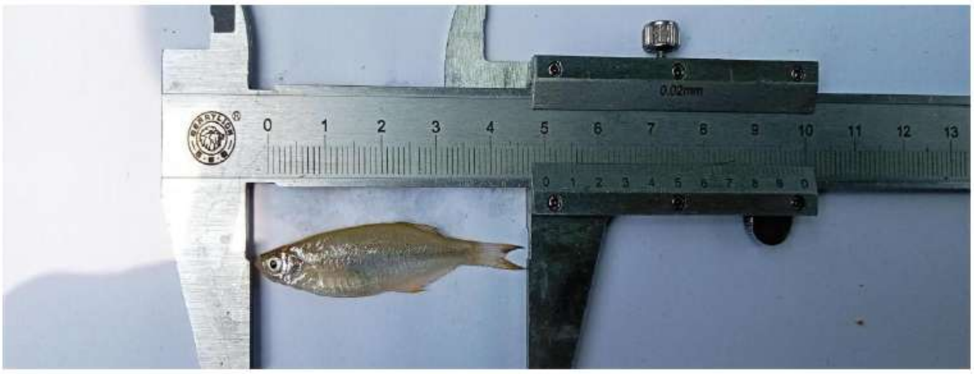

Photo 5: *Chela cachius*

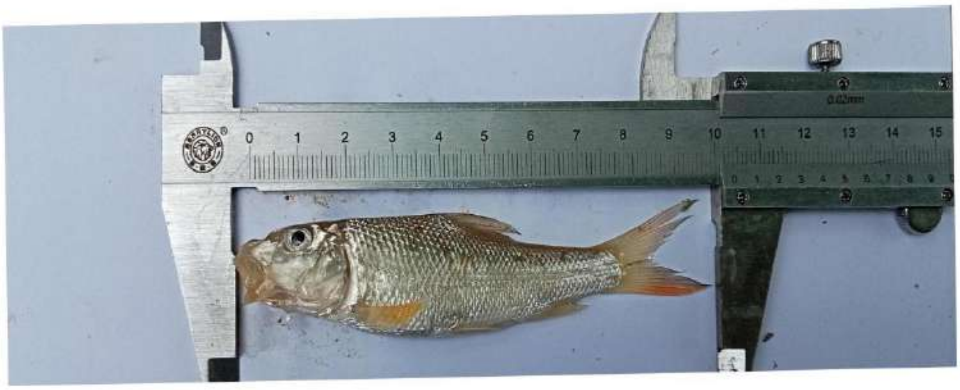

Photo 6: *Chagunius chagunio*

Photo Plate-III

Fish belonging to the order Cypriniformes

Photo 7: *Raiamas bola*

Photo 8: *Raiamas guttatus*

Photo 9: *Rasbora daniconius*

Photo Plate-IV

Fish belonging to the order Cypriniformes

Photo 10: *Salmostoma acinaces*

Photo 11: *Garra annandalei*

Photo 12: *Puntius sophore*

Photo Plate-V

Fish belonging to the order Cypriniformes

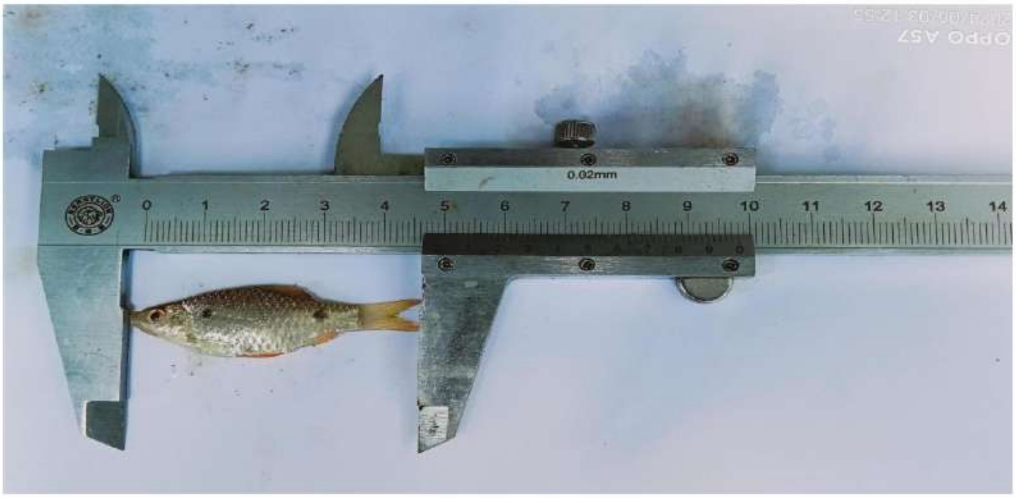

Photo 13: *Pethia ticto*

Photo 14: *Systomus sarana*

Photo 15: *Acanthocobitis botia*

Photo Plate-VI

Fish belonging to the order Siluriformes

Photo 16: *Sperata aor*

Photo 17: *Mystus bleekeri*

Photo 18: *Mystus cavasius*

Photo Plate-VII

Fish belonging to the order Siluriformes

Photo 19: *Gogangra viridescens*

Photo 20: *Heteropneustus fossilis*

Photo 21: *Pachypterus atherinoides*

Photo Plate-VIII

Fish belonging to the order Siluriformes

Photo 22: *Wallago attu*

Fish belonging to the order Anabantiformes

Photo 23: *Channa punctata*

Photo 24: *Channa orientalis*

Photo Plate-IX

Fish belonging to the order Anabantiformes

Photo 25: *Anabas cobajuis*

Photo 26: *Trichogaster lalius*

Photo Plate-X

Fish belonging to the order Anabantiformes

Photo 27: *Trichogaster fasciatus*

Fish belonging to the Order Synbranchiformes

Photo 28: *Macrognathus aral*

Photo Plate-XI

Fish belonging to the Order Synbranchiformes

Photo 29: *Mastacembalus armatus*

Fish belonging to the order Gobiiformes

Photo 30: *Glossogobius giuris*

Photo Plate-XII

Fish belonging to the order Mugiliformes

Photo 31: *Parambassis baculis*

Fish belonging to the order Beloniformes

Photo 32: *Xenentodon cancila*

Appendices

Appendix 1. Physico-chemical parameters of different stations and season

Water parameters	Seasons														
	Summer					Autumn					Winter				
	I	II	III	IV	V	I	II	III	IV	V	I	II	III	IV	V
Temperature (℃)	33	32	32.9	32.7	32	26	25.6	27	26.8	28	19	18.8	18.7	17.9	19
Velocity(m/s)	1.19	1.16	1.18	1.17	1.2	1.13	1.15	1.2	1.16	1.27	1.10	1.17	1.14	1.13	1.18
Transparency (cm)	89	66.5	55	86	40	87.5	67	56	85	39	88	66	54	86.5	37
pH	8.1	8.5	8.4	7.9	7.8	8.3	8.2	8.2	8.1	8.4	8.2	8.4	7.9	7.8	7.7
Free CO_2 (mg/L)	3.01	3.2	2.58	2.93	2.18	2.65	2.64	2.57	2.62	2.94	3.96	3.07	2.67	3.23	3.67
Dissolved Oxygen (mg/L)	5.67	5.7	6.8	5.9	5.71	6.48	6.89	6.08	6.5	6.7	6.08	8.1	6.8	7.25	6.87
Alkalinity (mg/L)	28	34	25	29	36	32	29	27	29.3	31.25	39	35	33	35.67	37
Hardness (mg/L)	56	58.3	52.6	59	55	59.06	47	54.9	59.4	58	52	54	52.5	51	55.5
TDS (ppm)	121	123	126	121	124	134	132	139	140	133	166	169	167	170	162
EC (µs/cm)	242	246	252	242	248	268	264	278	280	266	332	338	334	340	324

Appendix 2. Species code for DCA and RDA analysis and plotting

S.N	Species Code	Name of Species	Total
1	S1	*Acanthocobitis botia*	154
2	S2	*Anabas cobajius*	1
3	S3	*Barilius barila*	28
4	S4	*Barilius vagra*	23
5	S5	*Cabdio morar*	107
6	S6	*Chela cachius*	8
7	S7	*Chagunius chagunio*	4
8	S8	*Channa punctata*	22
9	S9	*Channa orientalis*	51
10	S10	*Garra annandalei*	14
11	S11	*Glossogobius giuris*	3
12	S12	*Gogangra viridescens*	62
13	S13	*Heteropneustes fossilis*	9
14	S14	*Macrognathus aral*	43
15	S15	*Mastacembalus armatus*	17
16	S16	*Mystus bleekeri*	38
17	S17	*Mystus cavasius*	80
18	S18	*Opsarius shacra*	1
19	S19	*Pachypterus atherinoides*	308
20	S20	*Parambassis baculis*	3
21	S21	*Pethia ticto*	66
22	S22	*Puntius sophore*	176
23	S23	*Raiamas bola*	1
24	S24	*Raiamas guttatus*	38
25	S25	*Rasbora daniconius*	1
26	S26	*Salmostoma acinaces*	42
27	S27	*Sperata aor*	3
28	S28	*Systomus sarana*	4
29	S29	*Trichogaster lalius*	5
30	S30	*Trichogaster fasciatus*	25
31	S31	*Wallago attu*	16
32	S32	*Xenentodon cancila*	1

Appendix 3. Permission Letter from the Department of Forestry and Soil Conservation, Government of Nepal

नेपाल सरकार
वन तथा वातावरण मन्त्रालय
वन तथा भू-संरक्षण विभाग

फोन नं. ४-२२५१७८, ४-२२०३०३
फ्याक्स: ४-२२७३७४

(कृपया पत्रोत्तरमा प्राप्त पत्र संख्या र मिति उल्लेख गर्नुहोला ।
बबरमहल, काठमाडौं, नेपाल

प्राप्त पत्र संख्या र मिति:-
पत्र संख्या:- ०८१/८२
च. नं.:- ३१०

मिति: २०८१/०६/०८

विषय: अनुसन्धान अनुमति सम्बन्धमा ।

श्री अजय झा,
सप्तरी, नेपाल ।

प्रस्तुत विषयमा Tribhuvan University, Central Department of Zoology, मा M. Sc. मा अध्ययनरत तपाईले "Spatiotemporal Variation of Fish Assemblages in Triyuga River, eastern Nepal" को विषयमा अध्ययन अनुसन्धानका लागि अध्ययन अनुमति उपलब्ध गराइदिनु हुन भनि मिति २०८१/०६/०६ गते यस विभागमा दिनु भएको निवेदन साथ प्रपोजल प्राप्त भयो। सो सम्बन्धमा कारवाही हुँदा उक्त अध्ययन अनुसन्धानबाट The gap on ichthyofaunal diversity, physio-chemical parameters and relationship between water quality parameters with fish species लगायतका विषयमा जानकारी प्राप्त हुने भएकोले प्रपोजलमा उल्लेखित Methodology (Cast Net and water parameters analysis) अनुसार तपसिलको शर्तहरुको अधिनमा रही डिभिजन वन कार्यालयसँग समन्वय गरि मिति सन् २०२४-०९-२४ देखि २०२५-०२-२८ सम्मका लागि अनुसन्धान गर्नु हुन निर्देशानुसार अनुरोध छ ।

शर्तहरु

१. अनुसन्धानकर्ताले वन ऐन २०७६ तथा वन नियमावली २०७९, राष्टिय निकुञ्ज तथा वन्यजन्तु संरक्षण ऐन, २०२९ र नियमावली २०३० तथा यस मातहतका नियमावलीहरुको पूर्ण पालना गर्नुपर्नेछ ।
२. अनुसन्धान कार्य डिभिजन वन कार्यालयसँगको समन्वयमा गर्नुपर्नेछ ।
३. सङ्कलित नमूनाको विश्लेषण Tribhuvan University, Central Department of Zoology को प्रयोगशालामा नै गर्नुपर्नेछ।
४. अनुसन्धानको क्रममा प्राप्त भएको जैविक विविधता संरक्षणसँग सम्बन्धित संवेदनशिल सूचनाहरु गोप्य राख्नु पर्नेछ अनाधिकृत रुपमा त्यस्ता सूचनाहरु कसैलाई पनि उपलब्ध गराउन पाइने छैन ।
५. अनुसन्धान कार्य समाप्त भए पश्चात एक प्रति रिपोर्ट/प्रतिवेदन (कागजी तथा विद्युतिय) यस विभागमा अनिवार्य रुपमा वुझाउनु पर्नेछ ।
६. तोकिएका शर्तहरुको पालना नगरिएमा विभागले कुनै पनि समयमा अनुसन्धान अनुमति रद्द गर्न सक्नेछ ।

(सुरेन्द्र प्रसाद अधिकारी)
वन अधिकृत

बोधार्थ
श्री डिभिजन वन कार्यालय, सप्तरी। : आवश्यक सहयोग तथा अनुगमनको लागि अनुरोध छ ।