SPATIO-TEMPORAL VARIATION IN FISH ASSEMBLAGE STRUCTURE OF UPPER RAPTI RIVER, MID-WESTERN NEPAL

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Master of Science in Zoology with special paper
Fish Biology and Aquaculture

Submitted To:
Central Department of Zoology
Institute of Science and Technology
Tribhuvan University
Kirtipur, Kathmandu
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September, 2022

DECLARATION

I hereby declare that the work presented in this thesis has been done by myself, and has not been submitted elsewhere for the award of any degree. All sources of information have been specially acknowledged by reference to the authors or institutions.

Date: 25 Aug 2022

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This thesis work submitted by **Hiramani Sharma** entitled **"SPATIO-TEMPORAL VARIATION IN FISH ASSEMBLAGE STRUCTURE OF UPPER RAPTI RIVER, MID-WESTERN 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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10	*Schizothorax labiatus*	II
11	*Schizothorax plagiostomus*	II
12	*Tor mosal*	II

LIST OF ABBREVIATIONS

Abbreviated form	Details of abbreviations
APHA	American Public Health Association
CO_2	Free carbon dioxide
DCA	Detrended Correspondence Analysis
DO	Dissolved Oxygen
FAO	Food and Agriculture Organization
NaOH	Sodium hydroxide
pH	Hydrogen ion concentration
RDA	Redundancy Correspondence Analysis

ABSTRACT

The spatial and temporal variations of fish assemblages in Nepalese rivers and streams is poorly known. The fish community and environmental factors of the Upper Rapti River were studied four times a year, spanning all seasons (summer, winter, autumn, and spring). The fish were agglomerated using a cast net with the assistance of local fishermen from three sample stations encompassing 15 kilometers. The current results indicate that the Upper Rapti River is habitat to 12 species representing 2 Orders, 4 Families, and 9 Genera. The most contributory fish species recorded from Upper Rapti River was *Barilius bendelisis* (18.34%) followed by *Schizothorax plagiostomus* (17.22%) and *Schizothorax labiatus* (15.31%). Kruskal-Wallis test was applied where p-value was observed 0.068 among seasons and 0.266 along stations so there was no significant difference in the abundance of fish among stations and seasons. Physical and chemical variables have a significant role in determining the condition of the fish community. Throughout a year, the mean Shannon-Weiner diversity index was 2.2 and species richness was maximum 2.2 during spring and least 1.6 during autumn. With a mean score of 0.9, species evenness was almost identical in all seasons. The RDA results showed that environmental elements such as pH, total hardness, free carbon dioxide, DO, and water temperature influence the fish community structure of the Upper Rapti River. The cluster analysis shows that the associations between fish assemblages and abundance of fishes. The research might contribute to the preservation of locally adapted fish in the Upper Rapti River as well as the advancement of information about the diversity of fish in Nepal.

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1. INTRODUCTION

1.1 General Background

Fish communities are geographically and temporally diversified, and biotic interactions like competition and predation are influenced by physical-chemical environmental factors (Gorman 1988). River fish assemblages vary spatially and temporally between several habitats (Adams et al. 2004). The organization of fish assemblages is influenced by spatial and temporal differences in salinity, temperature, dissolved oxygen, transparency, water current velocity, and tidal regime over the gradient (Sreekanth et al. 2020). Multiple variables working at various temporal and geographical dimensions have an impact on the spatial distributions of fish species and the resulting diversity of local assemblages (Presley et al. 2010, Pease et al. 2012). Therefore, understanding spatial and temporal variation is important for locating sources of assemblage control throughout the river and understanding time series data on fish assemblages (Schlosser 1990). The diversity of fish assemblages is influenced by a number of interrelated elements, including as the hydrologic regime, the geo-climatic area, the species composition, the balance of biotic and abiotic control, the history and frequency of disturbances in different types of channels, and many more (Grossman et al. 1998). Whereas the spatio-temporal variation of fish community structure depends on channelizes, incise, and sand bottom streams, temporal variation is considerable in warm water streams and in streams that have been anthropogenically disrupted (Schlosser 1982).

Environmental factors have a significant impact on the temporal fluctuation of communities (Perry et al. 2005), as well as the geographical distribution of species (Rouyer et al. 2008). Fishes are an essential part of the aquatic ecosystem, affecting the quantity and distribution of other creatures. They also serve as reliable indicators of the ecosystem's health and water quality (McCormick et al. 2000). Fish community has been observed to be influenced by habitat factors including water temperature, depth, water velocity, stream width, substrate, altitude, conductivity, dissolved oxygen, pH, free carbon dioxide, and climate (Magalhaes et al. 2002, Yu & Lee 2002, Gerhard et al. 2004, Kadye et al. 2008). Environmental factors may influence a population's spatial distribution and temporal dynamics at the same time, resulting in changes in the functional structure of populations

(Frelat et al. 2018). The existence of different species has a significant impact on how well ecosystems work and how well they are being conserved. As a result, fish communities play a crucial role as a biological indicator of changes in the river and lake ecology brought about by humans (Ru & Liu 2013).

Rivers are among the most heavily impacted ecosystems on the planet. They provide transportation, water supply, and electricity generating, as well as food and sewage clearance. Nepal is well known as the land of rivers, where it bears a huge potential of fisheries sector. A total of 6000 rivers and rivulets originating from Himalayas, Mahabharat and Siwalik mountain ranges (Gubhaju 2012) possessing extreme topography ranging in altitude from 60-8848 m from sea level and has huge water resources covers an area of 818,000 ha (about 2%) of the world's water resources (Rai et al. 2008). A huge number of fishermen and their families participate in the capture fisheries, which provides them with money and part-time work. Rivers are one of the key sources of the capture fishery. The major rivers of Nepal i.e. Koshi, Gandaki and Karnali along with other rivers/streams and scorers of lakes and reservoirs are the habitat of 232 species of fish from different climatic zones (Shrestha 2008). In addition to rivers, there are many lakes, ponds, reservoirs, etc. that offer sanctuary and feeding areas to freshwater fish.

Freshwater fish are among the most varied vertebrates and one of the most globally threatened species (Bruton 1995, Duncan & Lockwood 2001, Arthington et al. 2016). Also multiple human disturbances have an impact on the ecosystem, and fish assemblages are vulnerable to such changes (Fu et al. 2003, Liu et al. 2019). Over the past few decades, the structure of fish assemblages in the river system have decreased steeply due to pollution, harmful fishing practices, habitat modification, environmental degradation and barrier effects of dams and impact of other developmental activities (Assessment 2005). Globally, anthropogenic activities, urbanization, and industrialization have an impact on river ecosystems (Luo et al. 2022). The organization of the fish assemblage and biodiversity are altered by eutrophication, river-lake separation, and overfishing (Vonlanthen et al. 2012). Traditional monitoring techniques are often invasive and lack temporal and spatial resolution (Bolgan & Parmentier 2020). Freshwater fish use the river systems as spawning grounds, nurseries, and migration routes. Due to human activity and environmental changes, fish assemblages in rivers and lakes exhibit geographical and temporal variation (Jackson et al. 2001).

Numerous concerns put the variety and distribution of fish in rivers at danger. Impacts on the world's freshwater biodiversity are greater than those on its marine and terrestrial biodiversity (Dudgeon 2011, Liermann et al. 2012, Yang et al. 2016). Economic development is likely to degrade the native fish biodiversity due to increased anthropogenic activities (Gurung 2012). Native taxa are harmed or killed by invasive species due to competition, predation, habitat disturbance, hybridization, and the spread of parasites and illnesses (Strayer 2010, Li et al. 2012, Sheath et al. 2015). Similarly, tiny dams, land use, and water pollution are not managed properly on time, biodiversity would be severely lost as a result of ongoing environmental change, and extinctions might happen locally or globally (Arthington et al. 2016, Liu et al. 2019, Barbarossa et al. 2020, Díaz et al. 2021).

Freshwater ecosystems are already losing biodiversity at a faster pace than terrestrial systems (Tickner et al. 2020), and they are subject to a variety of anthropogenic influences such as climate change and the development of water resources on a global scale (Reid et al. 2019). Therefore, it is crucial for conservation, especially the preservation of sensitive ecosystems to understand the link between the environment and fish species distributions (Tickner et al. 2020).

1.2 Objectives of the study

1.2.1 General objective

The general objective of this study was to investigate the spatial and temporal variation of fish assemblage in Upper Rapti River, Mid-Western Nepal

1.2.2 Specific objectives

- To explore the spatio-temporal variation in species composition and abundance.
- To identify the factors affecting fish assemblage structure in Upper Rapti River.

1.3 Significance of study

The aquatic biodiversity of the world is decreasing rapidly as a result of extinction caused by habitat loss, pollution, introduction to exotic species, over exploitation and other anthropogenic activities. The Rapti River along with floods plains support wide range of biodiversity and services to society. The Rapti River system have vital fish genetic stocks and species complexes. These stocks are greatly affected by alteration of habitat, pollution and over exploitation of aquatic resources. For their proper conservation planning, there is a need to access species distribution in different geographic locations. Therefore, an effort has been made to investigate the fish diversity of the Upper Rapti River of Mid-Western Nepal, which is the first work in this study area.

2. LITERATURE REVIEW

2.1 Spatial variation of fish assemblage structure

As ecosystems are viewed over hierarchically structured and scale-dependent levels of variation and complexity, ecologists are increasingly looking at spatial and temporal variation to understand how various processes become important in determining population dynamics, species interactions, and assemblage structure (Naveh & Lieberman 2013). Variation in dispersion and invasion can alter population numbers, demography, and coadaptation, as well as food webs and assemblage structures, when different levels of the environmental hierarchy are crossed (Tonn et al. 1990, Endler 1991).

Several studies have been conducted on the geographical patterns of fish distributions at various spatial scales ranging from meters to hundreds or thousands of kilometers (Ault & Johnson 1998, Connell & Kingsford 1998, García-Charton & Pérez-Ruzafa 2001, Connell 2002). Protection from predators, ease of access to food, and preferences for nesting or spawning are just a few of the factors that might influence habitat choices (Stoffels et al. 2016, Schofield et al. 2018). Numerous studies have demonstrated the importance of habitat and physical factors, like as substrate and depth, in predicting the composition of fish species, with complex ecosystems supporting high fish biodiversity (Amoros & Bornette 2002, Zbinden & Matthews 2017, Lanés et al. 2018).

Spatial variation in natural resources may have been a key impetus for dispersal, migration and colonization of aquatic population (Flannery 2002). The variety and distribution of creatures such as vertebrates, invertebrates, and plants were shown to be connected to altitude. In general, species diversity decreases as altitude increases (Lomolino 2001). Altitude, river size, depth, water velocity, temperature, and habitat complexity are elements that influence the organization of fish assemblages in both temperate and tropical rivers (Tejerina-Garro et al. 2005). According to altitudinal variation, these structural alterations have a significant impact on diversity and fish assemblages (Jacobsen 2008). Therefore, various habitats can sustain functionally distinct fish assemblages, and hydrological connection is essential for preserving this variety at the watershed scale (Leigh & Sheldon 2009, Liu & Wang 2018, Rogosch & Olden 2019).

2.2 Temporal variation of fish assemblage structure

Fish are poikilothermic species, which means that their body temperature may change in response to environmental conditions. One of the most significant factors restricting fish variety in tropical and high altitude regions is temperature (Jacobsen 2008). The water temperature greatly influences on water chemistry and high water temperature cause the fluctuation in dissolved oxygen, hydrogen ion concentration, salinity and alkalinity of water (Fisher & Willis 2000). In addition, the aquatic life cycle, metabolism, and behavior of fish are all influenced by water temperature, and high water temperatures cause thermal stratification in rivers and lakes, which may result in less variety and uneven distribution of fish. Water temperature alters the rate of metabolism (Gillooly et al. 2001), growth and development (Wolter 2007), breeding (Mills & Mann 1985) and behaviour (Taniguchi et al. 1998). With an increase in altitude, the temperature drops. In comparison to lowland areas, the daily temperature variation is greater in the highlands (Buisson et al. 2008). Temperature of water decreases with increase in altitudinal gradient (Koster 2005, Sabater & Tockner 2009, Logez et al. 2012). Variation in precipitation is another significant element that affects fish assemblages in tropical streams and rivers. Just as the availability of food resources changes over both geography and time, so does its availability. Flood plains provide high levels of primary and secondary production as well as a variety of resources, which is beneficial for fish (Henderson 1990).

2.3 Environmental variables and fish assemblage structure

Variations in overall abundance and biomass throughout time reflect changes in the dominating species (Veiga et al. 2006). Fish assemblages were shown to be strongly connected to both water quality and habitat structure characteristics (Li et al. 2012). If there are seasonal or yearly shifts in fish faunal composition, they can be misconstrued for human influences, which would have a direct impact on management strategies and conservation efforts (Espírito-Santo et al. 2009). Seasonal fluctuations in abundance of some resident species were important in defining seasonal fish assemblages in the rivers (Idelberger & Greenwood 2005). Hossain et al. (2012) reported that the species abundance is maximum in summer and least in winter season. The quality of a stream and river system is mostly determined by the environmental and temporal effects on fish dispersion. Physico-chemical parameters including dissolved oxygen, pH, free carbon dioxide, conductivity, and water temperature are all associated to the distribution and assemblages of fish (Negi & Mamgain 2013).

Dissolved oxygen concentration and water temperature are inversely correlated. Therefore, seasonal fluctuation is more or less associated with fish composition (Li & Gelwick 2005, Trujillo-Jiménez et al. 2010). Due to seasonal differences in environmental factors, season has an effect on the distribution of fish composition in streams and rivers (Beugly & Pyron 2010). The main fish species in lowland warm water are those that can withstand high temperatures, such as *Aristichthys nobilis, Hypophthalmichthys molitrix, Ctenopharyngodon idella, Cyprinus carpio,* and *Labeo rohita* (Sharma 2008). In the mountainous region, carps such as *Tot tor, Tor putitora, Neolissochilus hexagonolepis, Labeo angra,* and others are found, although *Schizothorax* species predominate in cold-water streams in the highlands (Edds 1993).

Several research on fish diversity have been conducted in various locales of Nepal (Edds 1993, Sharma et al. 2001, Sharma 2008, Pokharel et al. 2018, Adhikari 2019, GC 2019, Ale 2020, Bhurtel 2020, Chaudhary et al. 2020, Limbu et al. 2020, Adhikari et al. 2021, Chapagain et al. 2021, Rajbanshi et al. 2021, Shrestha et al. 2021, Kumar et al. 2022, Neupane & Rajbanshi 2022), however, no one has studied the organization of the fish assemblage in the Upper Rapti River. As a result, the purpose of this study is to explore the spatio-temporal variation in the composition of the fish assemblage in the Upper Rapti River in Mid-Western Nepal.

3. MATERIALS AND METHODS

3.1 Study area

The current study was conducted in the Upper Rapti River in the Rolpa and Pyuthan districts of Lumbini Province. The research was carried out along a 15-kilometer stretch from Mijhing, Rolpa to Bhingree Bazar, Pyuthan. The river rises in the Mahabharat highlands to the north, merges with Madi, flows through Jhimruk, and then flows south through Dang and Banke districts. The research area extends from Bhingree, which is located at 28°08'43" N 82°42'50" E, to Mijhing, which is located at 28°14'16" N 82°43'00" E.

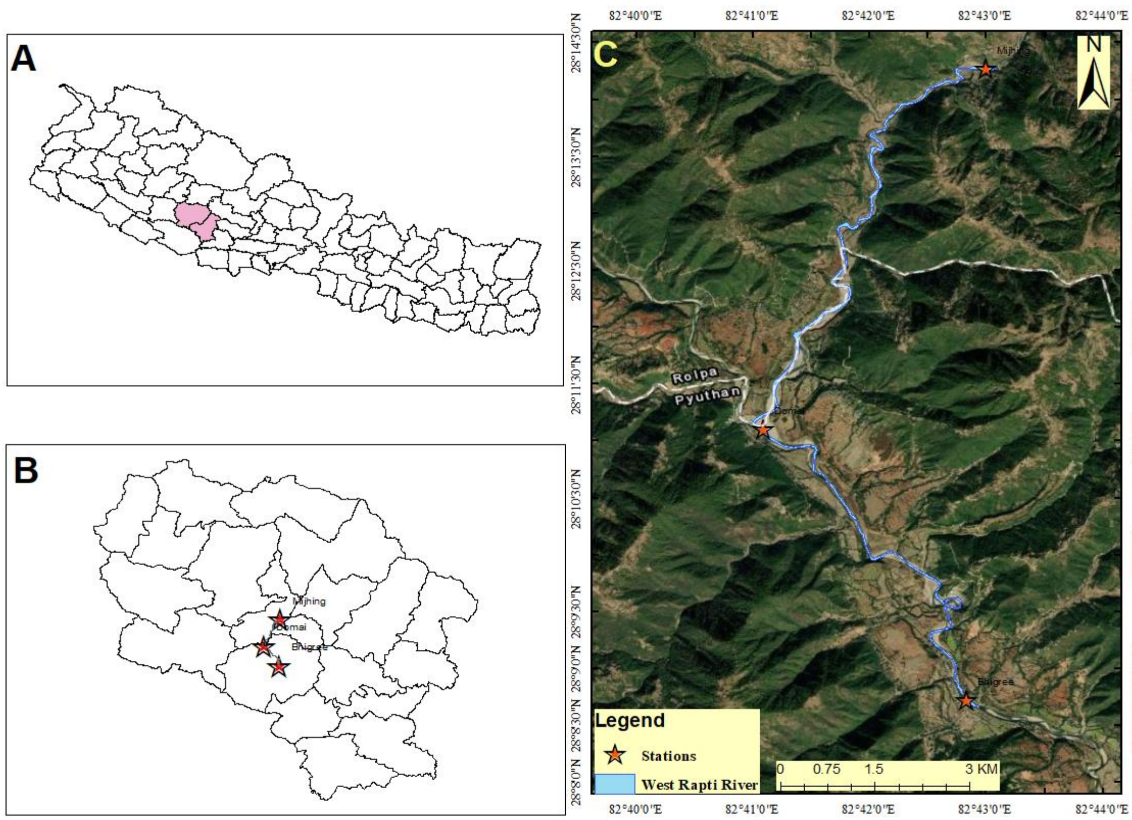

Figure 1 : Showing the study area

3.2 Study period

The field research was carried out between June 2021 and April 2022. Every sampling station was visited in the months of June, November, January, and April.

3.3 Selection of sampling sites

A preliminary survey was conducted before to commencing the research activity in order to set the study locations. The locations were chosen to represent the faunal distribution of the Upper Rapti River. The three sampling locations were chosen based on factors such as altitudinal variance, human habitation and confluence point. The three stations chosen are as follows:

Station I –Mijhing

Mijhing is located in Sunilsmriti Rural Municipality, Rolpa, around 4 kilometers from Sulichaur Bazar. It is located at 28°14'16" N 82°43'00" E and has an elevation of 761 m above sea level. The water velocity is greater in this location. This area is mostly made up of rocky structures with stones and gravels. Near the station, there is less human habitation and agricultural land.

Station II- Domai

Domai is located near Laltibang, on the border of the Rolpa and Pyuthan districts. It is located at 28°11'06" N 82°41'05" E and has a height of 656 m above sea level. People perform a funeral ceremony near the confluence. It is located 7 kilometers downhill from Station I. It is mostly composed of stones, pebbles, gravel, and sand. The nearest human habitation is roughly 500 meters away from the station.

Station III-Bhingree

Bhingree is located at 28°08'43" N 82°42'50" E and is 610 m above sea level. It is approximately 8 kilometers downhill from Station II. Human habitation is found on both banks of the river. It is mostly comprised of stones, gravel, and sand.

3.4 Collection and identification of fishes

For the current investigation, fish were obtained from each sample site by engaging a local fisherman, who used a cast net to capture the fish. Fish were collected using a cast net (10 mm mesh size, 3 m diameter, and 4.2 kg weight). Fish were sampled from 8 a.m. to 11 a.m. at each sampling location. For each station, the cast net was thrown 50-55 times at a distance of around 500m. Before preserving the fishes, the captured fishes were numbered and images were taken using a Samsung Galaxy A7 2018 smartphone. After photographing, the captured fish were stored in a 10% formalin solution. The samples were taken to the laboratory of Central Department of Zoology, Kirtipur, Kathmandu for identification. Fish identification was performed using the taxonomic keys of Shrestha (1981), Talwar & Jhingran (1991), Shrestha (1994), Jayaram (2013) and Shrestha (2019).

3.5 Analysis of environmental variables

Water samples of Upper Rapti River collected in the morning (08:00 to 11:00 a.m.) and tested once every three months during field trips. The physicochemical characteristics were investigated (APHA 1976, Adoni 1985, Tivedy & Geol, 1986).

3.5.1 Physical analysis of water

The existing meteorological conditions and the chemical composition affect the physical properties of water in any aquatic ecosystem.

3.5.1.1 Water temperature

The standard digital thermometer was used for recording the water temperature. The bulb of the thermometer was dipped inside the surface of water and reading was taken.

3.5.2 Chemical analysis of water

The chemical parameters was analyzed after (APHA 1976, Adoni 1985, Tivedy & Geol, 1986).

3.5.2.1 Hydrogen ion concentration

A digital pH meter (Hanna) was used to record the hydrogen ion concentration of water during the study period at every station of the Upper Rapti River.

3.5.2.2 Free carbon dioxide

A 50 ml sample was taken, and a few drops of the phenolphthalein indicator were applied to estimate the free carbon dioxide. The resultant colorless solution showed that carbon dioxide was present. Then, the solution was titrated to the slightly pink end point using a conventional alkali titrant (Sodium hydroxide 0.05N). The amount of free carbon dioxide in the water sample was determined using the formula below.

Free CO_2= $\frac{(ml \times N) of\ NaOH \times 1000 \times 44}{V}$

Where,

V=Volume of water sample taken (ml)

3.5.2.3 Dissolved Oxygen (DO)

Winkler's technique was used to calculate the amount of dissolved oxygen in the water. Every station's sample was taken and placed in a BOD container without bubbling. Manganese sulphate in the amount of two milliliters and alkaline iodine azide (KI) solution in the same amount were added, well agitated, and allowed to stand until it precipitated. To dissolve the brown ppt. that had accumulated on the bottom, two milliliters of Conc. Sulphuric acid were added to the solution. In the burette washed by the solution, 0.025N sodium thiosulphate was collected for titration. On the conical flask, 50 ml of the mixture was placed, and one or two drops of starch solution were added as an indicator. The solution was then titrated against a sodium thiosulphate solution until it turned colorless. The following formula was used to perform the computation.

$$DO\ (mg/l) = \frac{ml \times normality\ of\ titrant \times 8 \times 1000}{v2\ [\frac{v1-v}{v1}]}$$

Where,

v= Volume of $MnSO_4$ and KI added

v1=Volume of BOD bottle

v2=Volume of the part of the content titrated

3.5.2.4 Hardness

The total hardness of river water was determined using the EDTA titrimetric method.

$$\text{Total hardness as } CaCO_3\ (mg/l) = \frac{ml\ of\ EDTA \times 1000}{ml\ of\ sample}$$

3.6 Statistical analysis

3.6.1 Diversity Status

Seasonal and station wise Shannon Wiener diversity index (H'), Margalef's richness index (d) and Pielou's evenness index (J) was calculated in MS-Excel, 2013.

3.6.1.1 Shannon-Wiener Diversity index

The diversity of species was calculated by using Shannon-Weiner diversity index (Shannon & Weaver 1949)

Shannon Weiner diversity index was calculated as:

$$H' = -\sum \left(\frac{ni}{N}\right) \ln\left(\frac{ni}{N}\right)$$

Where,

ni = Importance values for each species is the number of individuals in each species, the abundance of each species.

N = Total importance value, the total number of individual observed.

3.6.1.2 Margalef's species richness index (d)

The species richness was calculated by using Margalef's Species richness (Margalef 1968). Margalef's richness index was designed as d, which was calculated as:

Margalef's species richness (d) = $\frac{S-1}{\ln N}$

Where,

S= Number of species

N= Number of individuals

3.6.1.3 Pielou's evenness index

The evenness of the species was calculated by using Pielou's species evenness (Pielou 1974). To calculate whether species are distributed evenly across seasons and across landscapes elements, evenness index was determined by using following formula.

Pielou's species evenness (E) = $\frac{H'}{\ln S}$

Where,

H′ = Shannon-Wiener's diversity index

S = Species richness is the number of species and is the simply a count of the number of different species in a given area.

3.6.2 Shapiro-Wilk normality test

The Shapiro-Wilk normality test was used whether or not the observed variables was normally distributed using vegan library in 'R' (Oksanen et al. 2020).

3.6.3 Kruskal-Wallis test

The Kruskal-Wallis test was used to determine whether or not there was statistically significant difference between the medians of three or more independent groups. The difference in the abundance of fish assemblages among stations and seasons was analyzed using Kruskal-Wallis test using vegan library in 'R' (Oksanen et al. 2020).

```
kruskal.test(Abundance ~ Seasons, data = df)

kruskal.test(Abundance ~ Station, data = df)
```

3.6.4 Multivariate analysis

The relation between species diversity and environmental variables was analyzed by redundancy analysis (RDA) method (Ter Braak 1988, Legendre et al. 2011) based on a linear response of species to environmental gradients by using vegan library in 'R' (Oksanen et al. 2020).

3.6.5 Cluster analysis

The relationship of spatial and temporal variation in fish assemblage was analyzed using cluster analysis. Similarly cluster analysis was done based on the abundance of fishes among seasons and stations using vegan library in 'R' (Oksanen et al. 2020).

4. RESULTS

4.1 Spatial and temporal variation of fish assemblage structure

4.1.1 Systematic position of fishes

Twelve different fish species were recorded belonging to 2 Orders, 4 Families and 9 Genera from three different stations of Upper Rapti River during four different seasons. The collected specimens were identified and given proper systematic position as described after Jhingran & Talwar (1991), Shrestha (1981), Jayaram (2013) and Shrestha (2019).

Table 1: Systematic position of fishes

S. N.	Order	Family	Scientific Name	Local Name
1.	Cypriniformes	Cyprinidae	*Garra gotyla gotyla* (Gray, 1830)	Nakato
2.			*Garra nasuta* (McClelland, 1838)	Nakato
3.			*Garra annandalei* Hora, 1921	Yaki
4.			*Barilius bendelisis* (Hamilton)	Faketa
5.			*Schizothorax labiatus* (McClelland, 1842)	Asala
6.			*Schizothorax plagiostomus* Heckel, 1838	Asala
7.			*Labeo caeruleus* Day, 1877	Gardi
8.			*Tor Mosal* (Hamilton, 1822)	Kade
9.			*Neolissochilus hexagonolepis* (McClelland, 1839)	Katle
10.		Nemacheilidae	*Schistura horai* (Menon, 1952)	Gadelo
11.		Botiidae	*Botia almorhae* Gray, 1831	Bai
12.	Siluriformes	Sisoridae	*Glyptothorax telchitta* (Hamilton, 1822)	Manero

4.1.2 Distributional pattern and frequency occurrence of fishes

A total of 627 individuals were enumerated which comprises of 12 species of fishes. The highest dominant species of Upper Rapti River was *Barilius bendelisis* (18.34%) followed by *Schizothorax plagiostomus* (17.22%). The lowest frequency was *Botia almorhae* (0.96%) followed by *Schistura horai* (2.07%).

Table 2 : Distribution and frequency occurrence of fishes of Upper Rapti River

S. N.	Name of fish	Summer			Autumn			Winter			Spring			Total	Relative Frequency
		I	II	III	I	II	III	I	II	III	I	II	III		
1	*Garra gotyla gotyla*	4	2	0	0	0	0	3	0	2	0	0	3	14	2.23
2	*Garra nasuta*	14	6	3	3	9	4	5	2	7	4	2	1	60	9.57
3	*Garra annandalei*	7	6	2	1	4	3	1	3	5	3	2	2	39	6.22
4	*Barilius bendelisis*	15	13	6	10	7	14	5	6	9	7	9	14	115	18.34
5	*Tor Mosal*	1	7	5	4	1	3	0	0	2	0	2	3	28	4.47
6	*Botia almorhae*	0	0	0	0	0	0	4	0	0	2	0	0	6	0.96
7	*Glyptothorax telchitta*	8	5	3	7	6	4	9	3	4	9	6	3	67	10.69
8	*Schistura horai*	4	0	0	0	0	0	6	0	0	3	0	0	13	2.07
9	*Schizothorax labiatus*	16	4	0	12	5	9	12	7	5	11	7	8	96	15.31
10	*Schizothorax plagiostomus*	19	8	1	11	13	7	7	9	11	9	8	5	108	17.22
11	*Labeo caeruleus*	2	5	2	0	3	7	0	0	0	0	5	3	27	4.31
12	*Neolissochilus hexagonolepis*	5	3	8	2	5	2	5	4	2	3	7	8	54	8.61
	Total	95	59	30	50	53	53	57	34	47	51	48	50	627	100.00

4.1.3 Order wise distribution of fish in Upper Rapti River

The study revealed that Order Cypriniformes was the most dominant order followed by Order Siluriformes as indicated in figure 2.

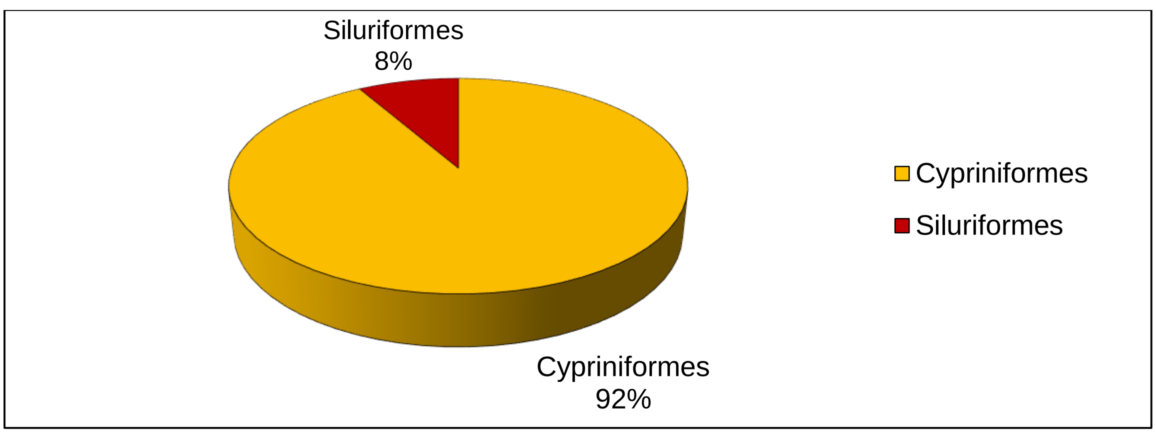

Figure 2: Order wise distribution in Upper Rapti River

4.1.4 Family wise distribution of fish in Upper Rapti River

The results showed that the family Cyprinidae had the most abundant fish species, followed by the families Nemacheilidae, Botiidae, and Sisoridae (8.33%) equally, as indicated in the figure 3.

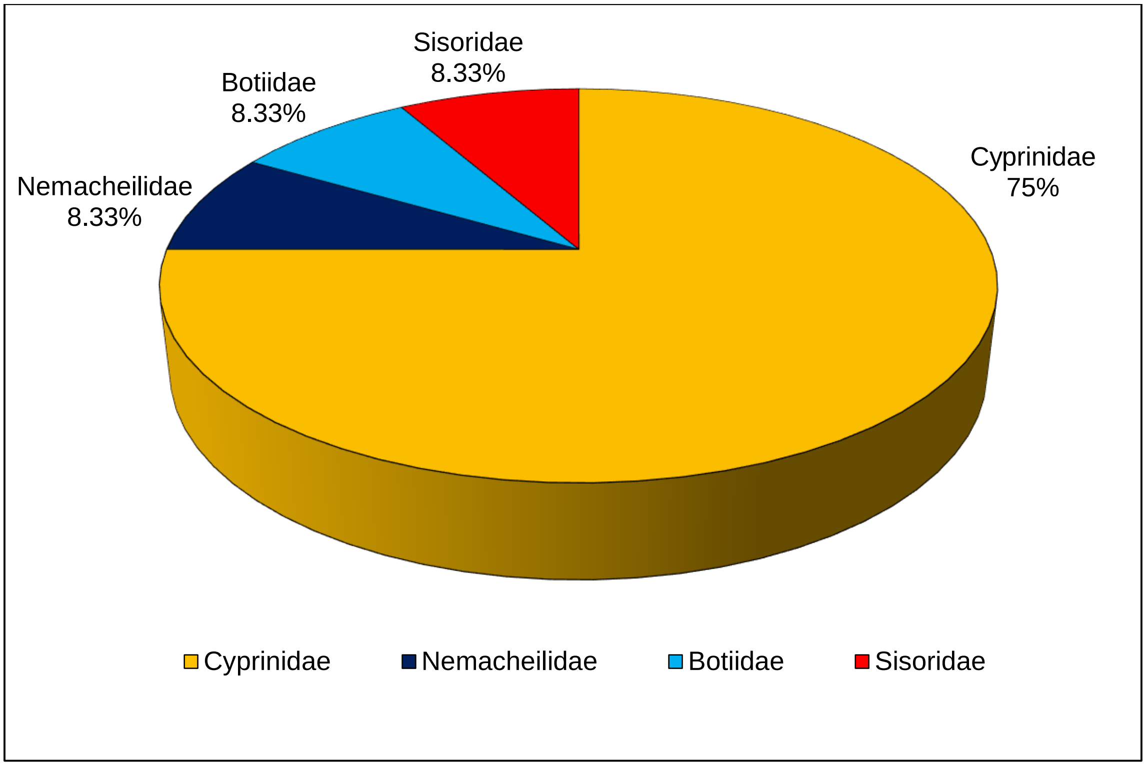

Figure 3: Family wise distribution of fish species in Upper Rapti River

4.1.5 Diversity status of fish

The Upper Rapti River's fish assemblage varied spatially, with the greatest number of individuals being found at Station I and the smallest at Station III, which was close to human influence and fishing pressures. The values of Shannon-Wiener diversity index (H'), Simpson's index, Margalef's richness (d) and Pielou's evenness index (J) were calculated according to stations. The highest Shannon Weiner diversity index 2.2 was found equally in station I and III, whereas in station II it was found a bit less i.e. 2.1. No significant difference was found in the mean Shannon-Wiener diversity among the seasons. Similarly, species richness was maximum 2.2 during spring and least 1.6 during autumn. Species evenness was almost equal in all the stations with mean value 0.9. (fig. 4)

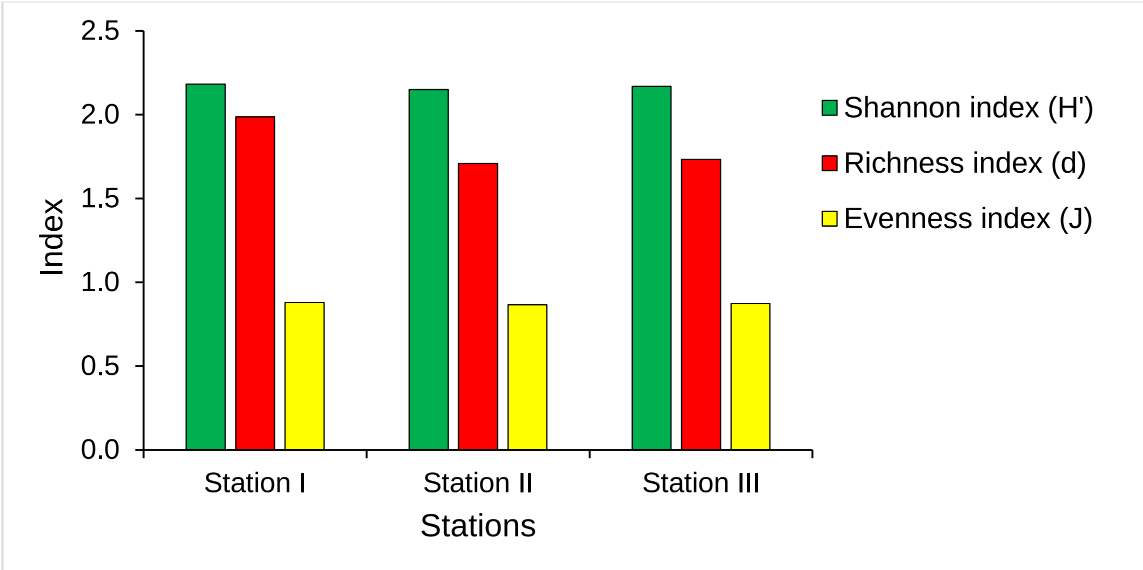

Figure 4: Spatial variation of fish diversity of Upper Rapti River

The Upper Rapti River's fish assemblage varied temporally, with the highest number of individuals being found during summer season and lowest during winter season. The values of Shannon-Wiener diversity index (H'), Margalef's richness (d) and Pielou's evenness index (J) were calculated according to seasons. The highest Shannon Weiner diversity index 2.3 was found during summer, whereas during autumn it was found least i.e. 2.1. No significant difference was found in the mean Shannon Weiner diversity among the stations. Similarly, species richness was maximum 2.0 at station I and least 1.7 at both station II and III. Species evenness was almost equal during all seasons with mean value 0.9. (fig. 5)

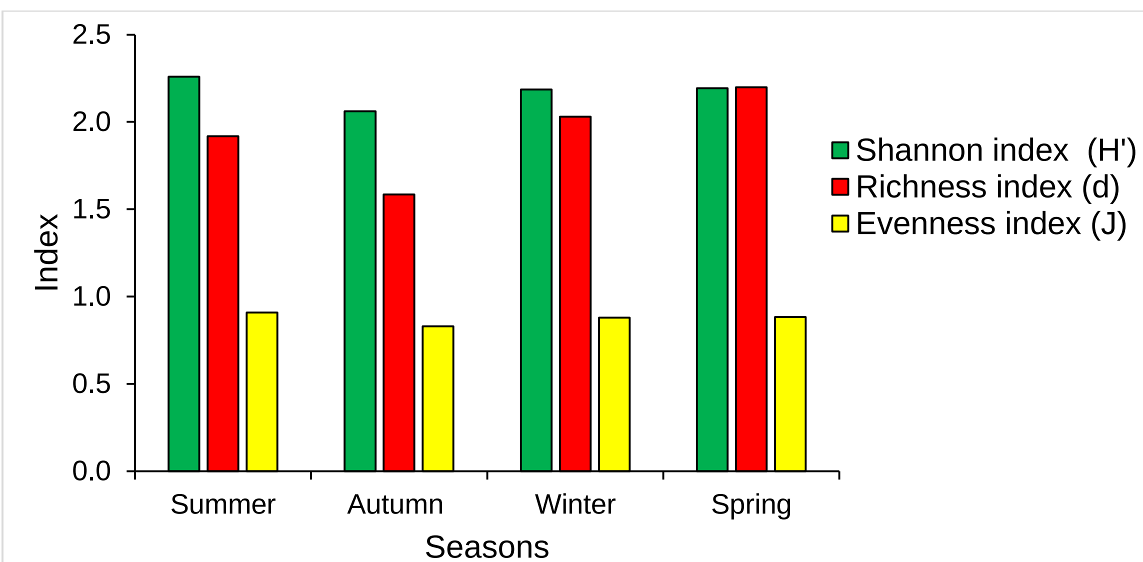

Figure 5: Temporal variation of fish diversity of Upper Rapti River

4.2 Spatial and temporal variation of the environmental variables

Physico-chemical parameters like water temperature, pH, dissolved oxygen, free carbon dioxide and hardness of water were measured during the research process.

4.2.1 Physical parameters of water

4.2.1.1 Water temperature

The highest range of temperature was recorded at station II during summer season with value of 21.5°C and lowest was recorded at station I during winter season with value of 12.8°C. The average temperature of station I, station II and station III were 17.3°C, 17.9°C and 18°C, respectively. The average value of temperature recorded during the whole study period was 17.75°C. (fig. 6)

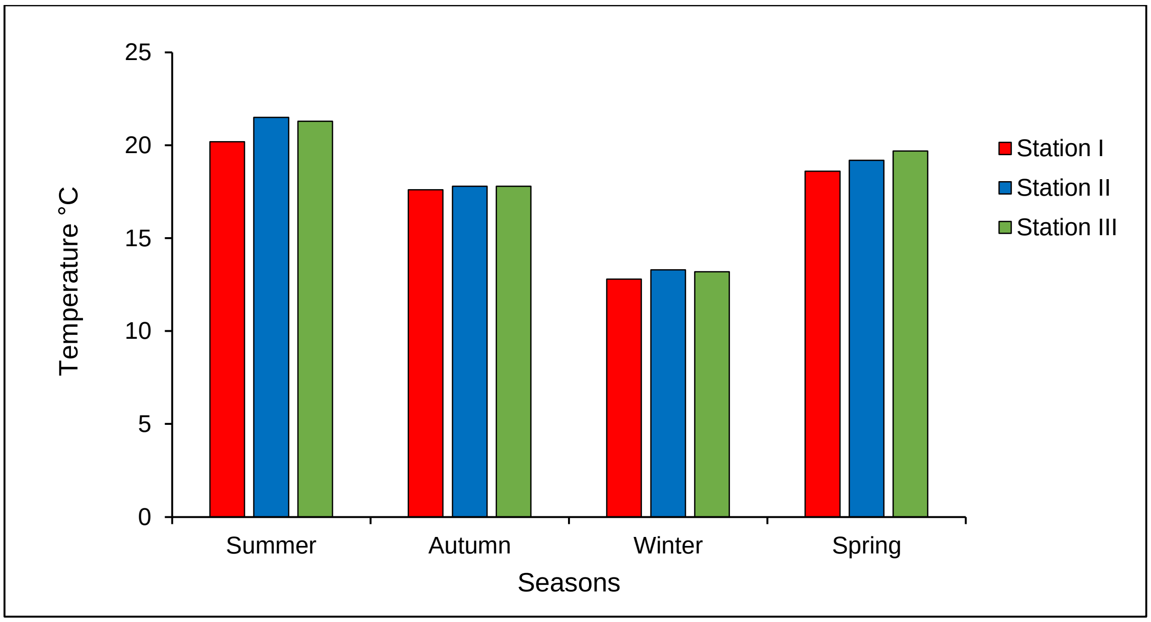

Figure 6: Variation in temperature of Upper Rapti River

4.2.2 Chemical parameters of water

4.2.2.1 Hydrogen ion concentration (pH)

The pH of water of Upper Rapti River was noted slightly alkaline in all stations. The highest value of pH was recorded 9.5 from station III during autumn season and lowest was recorded 7.8 from station I during same season. The average pH of water from station I, station II and station III were 8.1, 8.7 and 8.6, respectively. The average value of pH recorded during the whole study period was 8.4. (fig. 7)

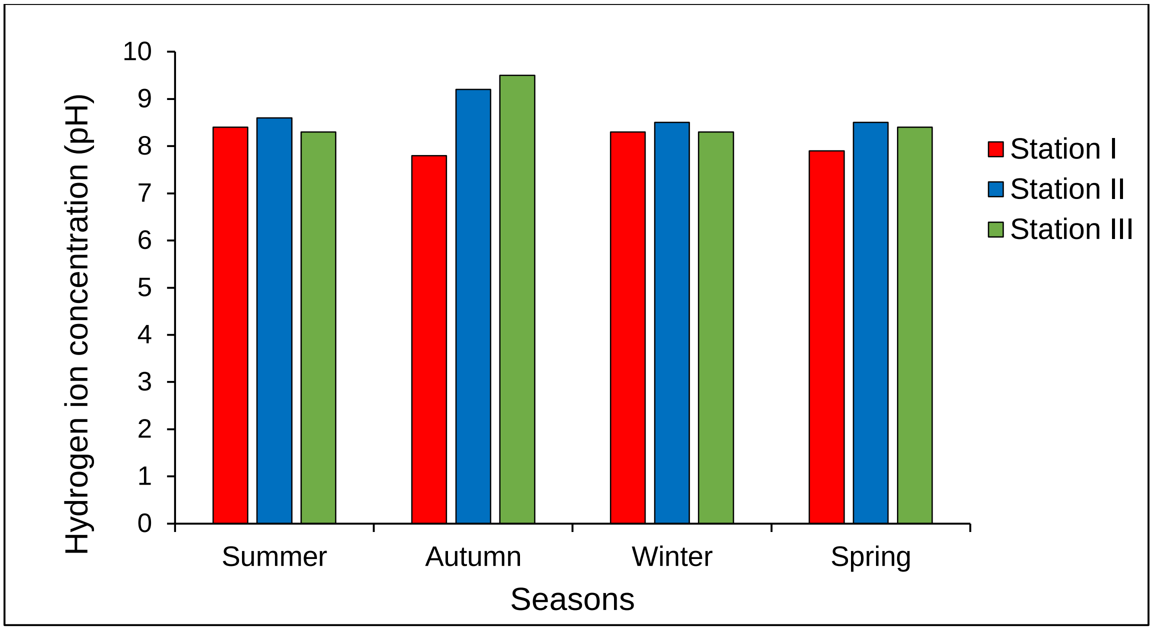

Figure 7: Variation in pH of Upper Rapti River

4.2.2.2 Free Carbon dioxide

The highest value of free carbon dioxide was recorded 2.5mg/l during summer season from station II and lowest value was 1.1mg/l during winter season from station I. The average free carbon dioxide was recorded 1.8mg/l. (fig. 8)

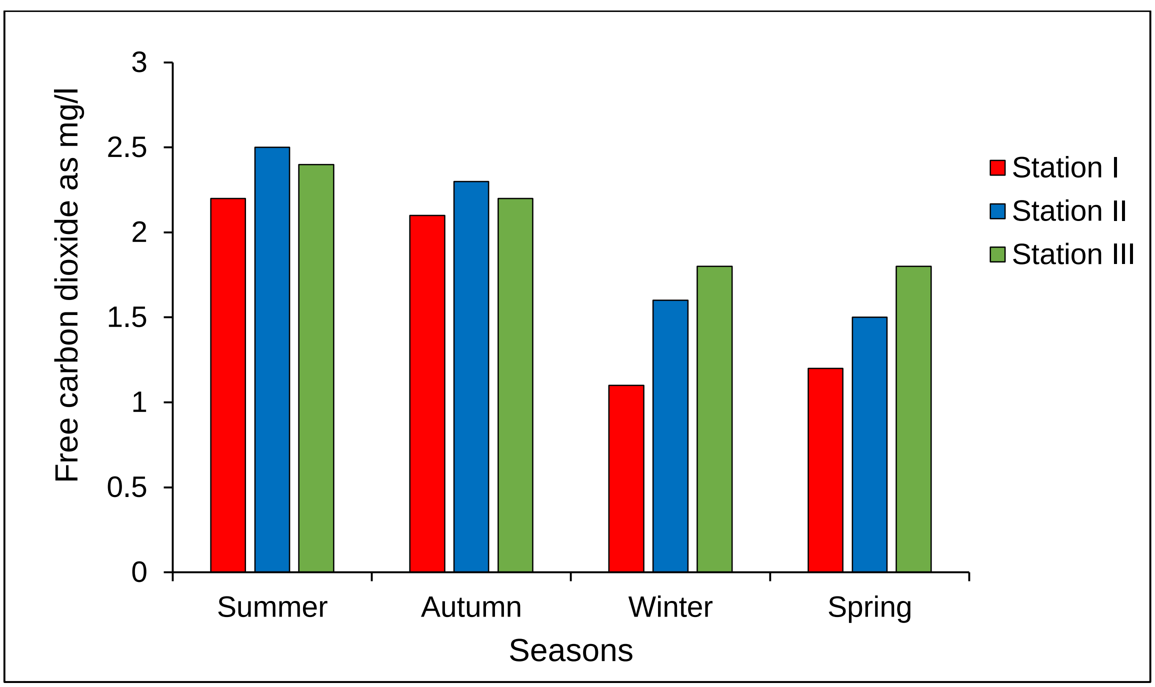

Figure 8: Variation in free carbon dioxide of Upper Rapti River

4.2.2.3 Dissolved Oxygen

The highest amount of dissolved oxygen was recorded 9.6mg/l from station I during winter season and lowest 8.0mg/l from station III during summer season. The average value of dissolved oxygen was found to be 8.8mg/l during research period. (fig. 9)

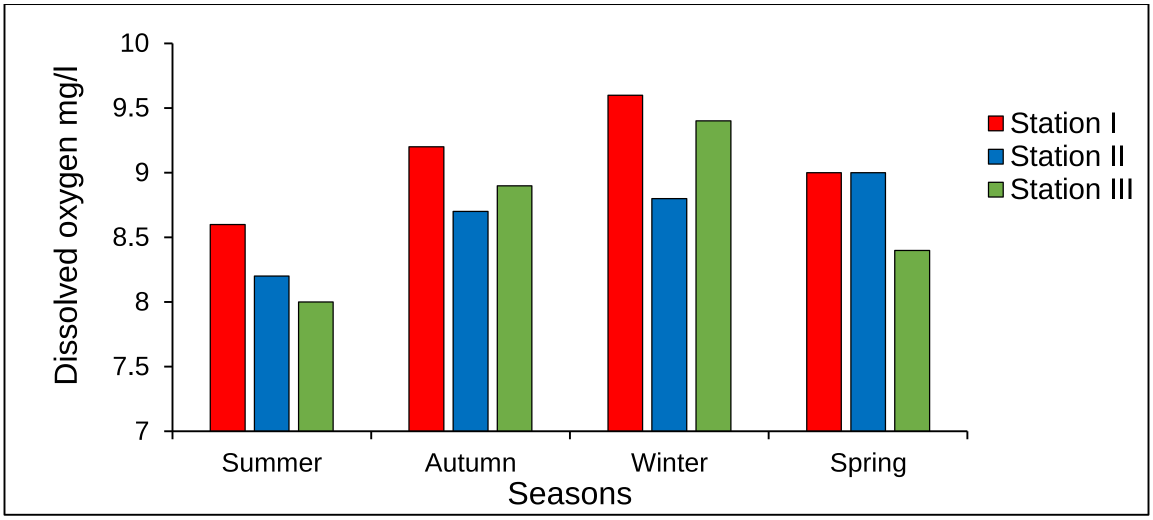

Figure 9: Variation in dissolved oxygen of Upper Rapti River

4.2.2.4 Total hardness (mg/l as $CaCO_3$)

The maximum value of total hardness as $CaCO_3$ was recorded 151.2mg/l from station III during spring season and minimum value of 104.0mg/l from station I during winter season. The average amount of total hardness as $CaCO_3$ was recorded 127.4mg/l during study period. (fig. 10)

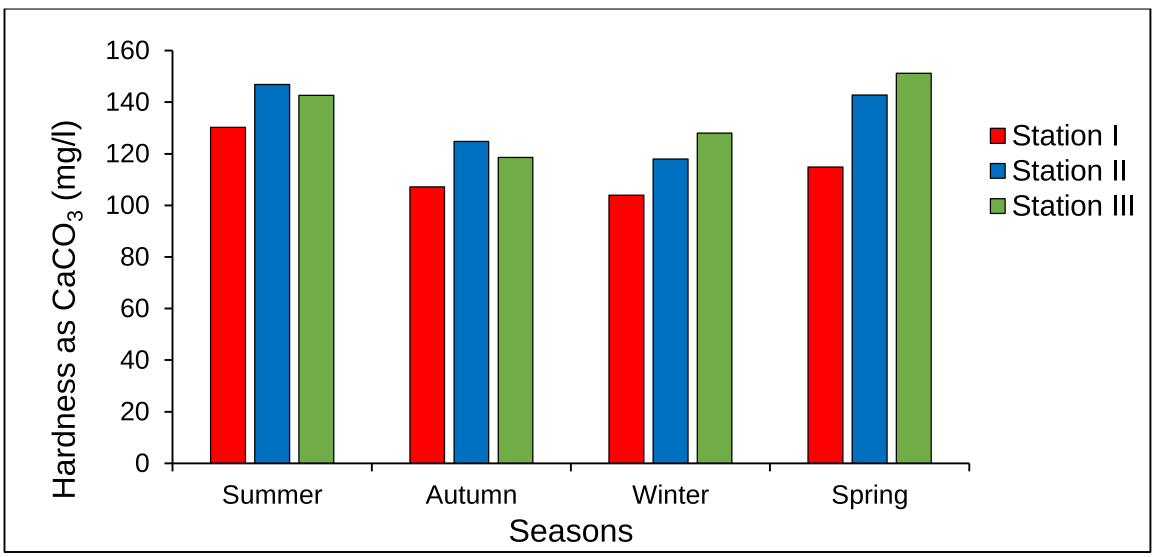

Figure 10: Variation in total hardness of Upper Rapti River

4.3 Relationship between different seasons, stations, environmental variables and abundance of fish species

4.3.1 Spatial and temporal distribution and abundance of fish

The Shapiro-Wilk normality test provided the p-value of 0.01087 which is less than 0.05 so, the observed variables was not normally distributed. Therefore, Kruskal-Wallis test was applicable where p-value was observed 0.068 among seasons and 0.266 among stations which exceeds 0.05 so there was no significant difference in the abundance of fish among stations and seasons.

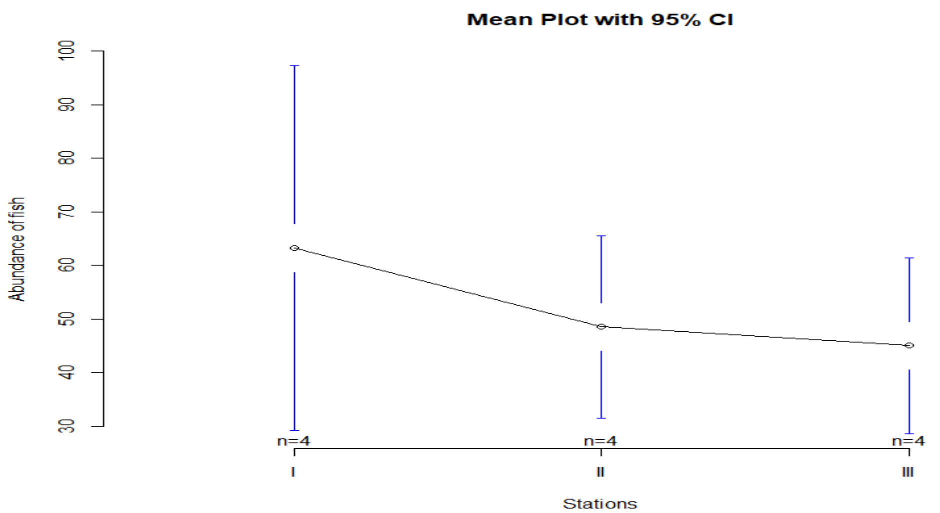

Figure 11: Abundance of fish among stations

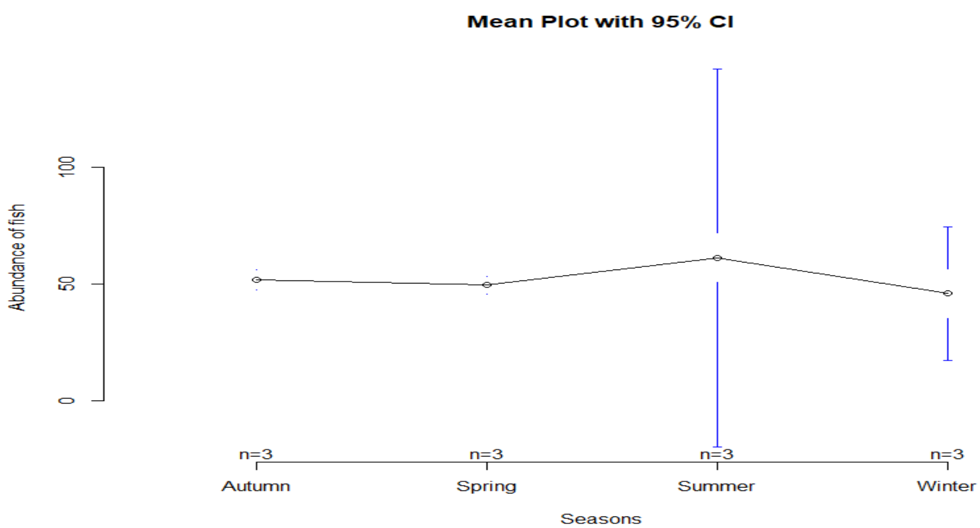

Figure 12: Abundance of fish among seasons

4.3.2 Ordination

The first axis of the Detrended Correspondence Analysis (DCA) had an axis length of 1.49, and the second axis had an axis length of 0.61. Similar to that, the data matrix 's eigenvalues were 15%. RDA's application was therefore reasonable.

Table 3 : DCA summary

	DCA1	DCA2	DCA3	DCA4
Eigen values	0.1520	0.03828	0.027376	0.021484
Decorna values	0.1645	0.02455	0.008082	0.001254
Axis length	1.4998	0.061279	0.589542	0.487820

4.3.3 Fish species, stations, seasons and environmental relationship

The result obtained from redundancy analysis (RDA) was plotted in figure 13. Water temperature (wate_temp), pH and free carbon dioxide (Free_carb) were found significantly correlated. Whereas, dissolved oxygen (Diss_oxyg) and total hardness of water (Wate_hard) had strong negative realtion to each other. Occurrence of *Glyptothorax telchitta, Schizothorax labiatus, Schizothorax plagiostomus* and *Schistura horai* shows positive correlation with dissolved oxygen. *Labeo caeruleus, Barilius bendelisis*, *Tor mosal, Garra annandelei* and *Garra nasuta* were positively correlated with pH, water temperature and free carbon dioxide whereas *Botia almohrae* had strong negative relation with these variables. *Neolissochilus hexagonolepis* shows positive correlation with station II and total hardness of water. *Botia almohrae* shows neither positive nor negative relation with total hardness and dissolved oxygen.

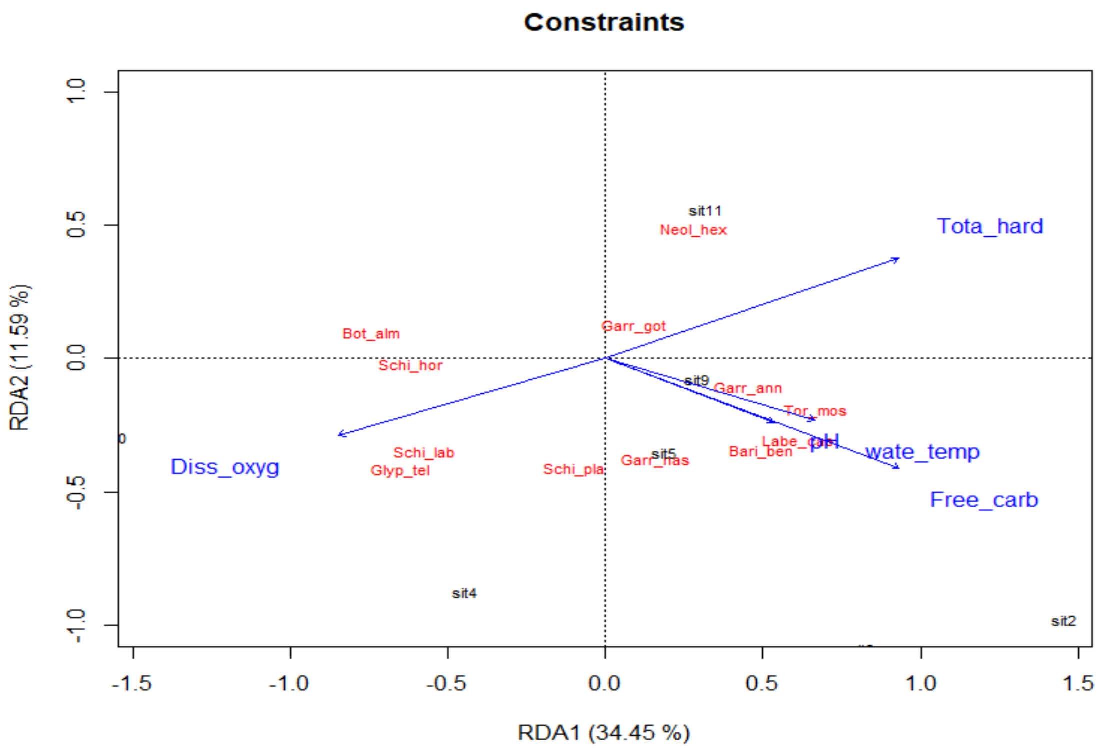

Figure 13: RDA analysis of species abundance, stations and environmental variables

Abbreviation: Garr_got = *Garra gotyla gotyla*, Garr_nas = *Garra nasutsa*, Garr_ann = *Garra annandalei*, Bari_ben = *Barilius bendelisis*, Tor_mos = *tor mosal*, Boti_alm = *Botia almorhae*, Glyp_tel = *Glyptothorax telchitta*, Schi_hor = *Schistura horai*, Schi_lab = *Schizothorax labiatus*, Schi_plag = *Schizothorax plagiostomus*, Labe_cae = *Labeo caeruleus*, Neol_hex = *Neolossochilus hexagonolepis*

Diss_oxyg = Dissolved oxygen, Tota_hard = Total hardness, wate_temp = Water temperature, Free_carb = Free carbondioxide

4.3.4 Cluster Analysis

4.3.4.1 Cluster analysis comparing sampling stations and seasons

The cluster dendrogram (Fig 14) shows the relationship among stations and seasons on the basis of abundance of fish assemblages. 1, 7, 10 form a significant cluster which shows the similar fish assemblage from station I during three seasons i.e. summer, winter and spring. Similarly, in the middle, 11 and 12 form a significant cluster between station II and III during spring season. Also, 5 and 9 forms separate cluster between station II during autumn and station III during winter based on the assemblage structure of fishes in Upper Rapti River.

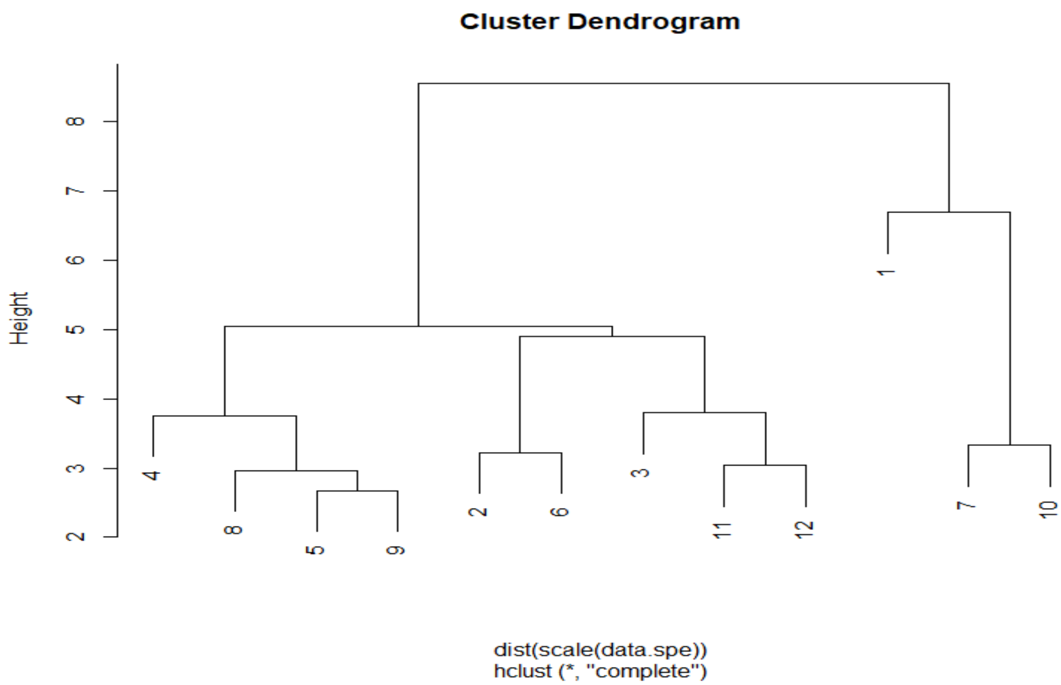

Figure 14: Dendrogram of cluster analysis comparing sampling stations and seasons on the basis of fish assemblage

Abbreviation: 1=Summer Station I, 2=Summer Station II, 3=Summer Station III
4=Autumn Station I, 5=Autumn Station II, 6=Autumn Station III
7=Winter Station I, 8=Winter Station II, 9=Winter Station III
10=Spring Station I, 11=Spring Station II, 12=Spring Station III

4.3.4.2 Cluster analysis comparing fish species on the basis of fish assemblage

The cluster analysis comparing fish species on the basis of fish assemblage was shown in figure 15. *Botia almorhae* and *Schistura horai* formed disparate cluster in the left part of cluster dendrogram. These two species were observed only from station I and have the least frequency among all species. *Barilius bendelisis, Schizothorax labiatus* and *Schizothorax plagiostomus* form another cluster as these species were most abundant species among all seasons and stations. *Garra annandelei* and *Garra nasuta* were also observed almost in all seasons and stations forming the significant cluster. Accordingly, *Tor mosal* and *Labeo* *caerelus* have the similar distribution and frequency forming separate cluster in the right side of cluster dendrogram.

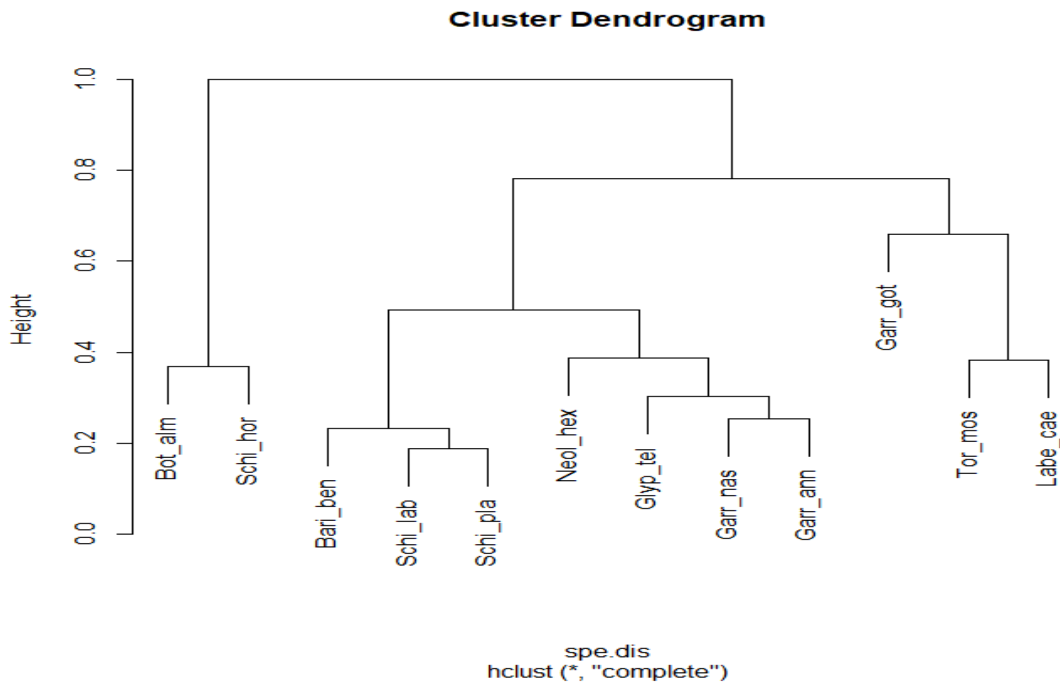

Figure 15: Dendrogram of cluster analysis comparing fish species on the basis of fish assemblage

Abbreviation: Garr_got = *Garra gotyla gotyla*, Garr_nas = *Garra nasutsa*, Garr_ann = *Garra annandalei*, Bari_ben = *Barilius bendelisis*, Tor_mos = *tor mosal*, Boti_alm = *Botia almorhae*, Glyp_tel = *Glyptothorax telchitta*, Schi_hor = *Schistura horai*, Schi_lab = *Schizothorax labiatus*, Schi_plag = *Schizothorax plagiostomus*, Labe_cae = *Labeo caeruleus*, Neol_hex = *Neolossochilus hexagonolepis*

5. DISCUSSION

5.1 Spatial variation in fish assemblage structure

River systems offer a variety of habitats for many native fish species. 12 fish species were collected from three sampling stations of Upper Rapti River, representing 2 Orders, 4 Families and 9 Genera. During the research period, 627 fishes were captured. This suggested that the Upper Rapti River serves as a primary source of livelihood and nutrition for local fishermen and fishing communities.

Among two orders Cypriniformes and Siluriformes occupy 92% and 8% respectively. Shrestha (2019) reported 252 fish species from Nepal, encompassing 15 Orders, 35 Families and 104 Genera. Cypriniformes is the most dominant order with 135 species. On this basis, we may generally conclude that Nepali rivers provide suitable habitat for the Order Cypriniformes. The current study also supported the result of Shrestha (2019). Out of 12 species, 11 species belong to Order Cypriniformes. The fish species of the Cyprinidae family (75%) was most dominated fish species followed by Nemacheilidae, Botiidae and Sisoridae (8.33%) equally. Edds (1993), Sharma and Shrestha (2001) had found that Cyprinidae was the most dominant family in Kaligandaki and Tinau River respectively. Similarly, Oli (2016), Chaudhary et al. (2020) also reported the Cyprinidae family was dominant from West Rapti River which was similar in finding with current study.

The spatial distribution of fishes in the Upper Rapti River was discovered to differ substantially. The least abundant species was *Botia almorhae* (0.96%) followed by *Schistura horai* (2.07%) and *Garra gotyla gotyla* (2.23%). Throughout the research period, *Botia almorhae* and *Schistura horai* were not found at stations II and III. It might be due to high temperatures, low elevation, or human interference. The most dominated fish species recorded from Upper Rapti River was *Barilius bendelisis* (18.34%) followed by *Schizothorax plagiostomus* (17.22%) and *Schizothorax labiatus* (15.31%). *Barilius bendelisis* had the highest frequency of capture among fishes collected primarily from station III rather than stations I and II. Station I was dominated by *Schizothorax labiatus* and *Schizothorax plagiostomus*. This might be because of the lower water temperature, high elevation and rocky environment. Other species observed at all three sites included

Garra gotyla, Garra nasuta, Garra anandelei, Tor mosal, Glyptothorax telchitta, Labeo caeruleus, and *Neolissochilus hexagonolepis.* Station I recorded the highest number of individuals among the three sites though there is no significant difference in the abundance of species along the stations.

According to Shannon-Weiner diversity index values, the biodiversity index values obtained from the current study were not very high and did not accurately depict the variations across the stations. In all stations, the mean Shannon-Weiner diversity index was 2.2. Similarly, species richness index was observed maximum value of 2.0 at station I whereas 1.7 equally at both station II and III. With a mean score of 0.9, species evenness was almost identical in all three locations.

5.2 Temporal variation in fish assemblage structure

Summer had the highest number of individuals among the four seasons though there is no significant difference in the abundance of fish species among seasons. In terms of temporal change, most of the fishes were reported from all four seasons. The peak number of fishes was observed in the summer, while the lowest number was observed in the winter which supported the finding of Hossain et al. (2012). *Botia almorhae* was only observed throughout the winter and spring. Similarly, *Garra gotyla gotyla* and *Schistura horai* were not recorded in autumn season. *Barilius bendelisis, Schizothoreax labiatus,* and *Schizothorax plagiostomus* were the three most prevalent species in the Upper Rapti River throughout the research period and were visible in all four seasons. *Garra nasuta* was the most prevalent of the three *Garra* species observed, followed by *Garra annandelei* and *Garra gotyla gotyla*. *Labeo caeruleus* was found approximately equally throughout the summer, autumn, and spring, but not in the winter. *Glyptothorax telchitta* was seen throughout the year. *Tor Mosal* had the most individuals during the summer and the fewest during the winter, whereas Neolissochilus *hexagonolepis* had the most individuals during the spring and the fewest during the autumn season.

During the summer season, station II recorded the maximum water temperature (21.5°C), while station I recorded the lowest temperature (12.8°C). As a result, water temperature is positively connected with fish assemblages and influences fish species distribution.

According to Shannon-Weiner diversity index values, the biodiversity index values obtained from the current study were not very high and did not accurately depict the variations among seasons. Throughout a year, the mean Shannon-Weiner diversity index was 2.2. Similarly, species richness was obtained maximum during spring with value 2.2 and least value of 1.6 during autumn. With a mean score of 0.9, species evenness was almost identical in all seasons.

5.3 Environmental variables and fish assemblage structures

The quantity of aquatic life was influenced by the physicochemical features of the aquatic environment. Water temperature, free carbon dioxide, dissolved oxygen, and pH are all connected with fish abundance, and these factors vary according to season and altitude (Negi & Mamgain 2013, Schofield et al. 2018). Similarly, dissolved oxygen is critical for aquatic life survival, influencing the distribution, diversity, physiology, and behavior of fishes (Pokharel et al. 2018). According to Doudoroff and Shumway (1970), for aquatic wildlife to survive, the quantity of dissolved oxygen should be more than 5mg/l. Dissolved oxygen of current study was recorded 9.6mg/l from station I during winter season and lowest 8.0mg/l from station III during summer season. The average value of dissolved oxygen was recorded 8.8mg/l during research period.

The bulk of the carbon dioxide in water is produced by organisms' respiration and the decomposition of organic waste. According to Tix et al. (2017), rising CO_2 levels due to climate change can harm fish homing to natal settings while also impairing their capacity to detect predators and operate aerobically. Free carbon dioxide levels in the current research ranged from 1.1mg/l to 2.5mg/l, indicating that the river water supports fish life. In all sites, the pH of the water in the Upper Rapti River was found to be slightly alkaline similar to the result reported by Ale (2020) from Dobhan Khola. The station III in the autumn recorded a maximum pH value of 9.5, while station I in the same season recorded a minimum pH value of 7.8. According to West et al. (1997), a pH level between 6.5 to 9.5 is ideal for optimal performance; anything less or more than this range is stressful.

The Shapiro-Wilk normality test indicates the observed variables were not normally distributed. Hence, Kruskal-Wallis test was applicable where p-value among stations and seasons were 0.266 and 0.068, respectively which exceeds the value of 0.05. So it is concluded that there is no any significant difference in the abundance of fish assemblages among stations and seasons. However, the mean plot with 95% CI shows that station I has slightly higher abundance of fish assemblages than station II and III as shown in figure 11. Also, while observing through seasons, summer season was observed slightly highest among other seasons as shown in figure 12. It might be because of similar environmental variables among stations throughout the year.

According to local fishermen, this river had fishes such as *Bangana dero, Bagarius bagarius, Monopterus cuchia* and *Anguilla bengalensis*. However, we were unable to collect these specimens within the research time. There might be several reasons for this, including unsuitable gear selection, habitat degradation, trafficking of rocks, stones, and sand, climate change, and day-to-day fluctuations in water volume.

Physical and chemical variables have a significant role in determining the condition of the fish community (Li et al. 2012). The RDA results showed that environmental elements such as pH, total hardness, free carbon dioxide, DO, and water temperature influence the fish community structure of the Upper Rapti River. The ordination plot (Fig 13) showed environmental variables of water temperature, pH and free carbon dioxide were significantly correlated. Dissolved oxygen and total hardness of water had strong negative correlation. *Glyptothorax telchitta, Schizothorax labiatus*, *Schizothorax plagiostomus* and *Schistura horai* are positively correlated with dissolved oxygen. *Botia alomorhae* observed from station I does not show any significant positive relation with environmental variables. Total hardness is associated with *Neolissochilus hexagonolepis* and *Garra gotyla gotyla*. *Barilius bendelisis, Tor mosal, Labeo caereleus*, *Garra annandalei* and *Garra nasuta* are positively correlated with pH, water temperature and free carbon dioxide. The previous studies, such as Yu & Lee (2002), Kadye et al. (2008), Mishra & Baniya (2016) have also stated that external factors play a key role in influencing the structure of fish communities.

6. CONCLUSION

6.1 Conclusion

A total of 627 individuals were enumerated which comprises of 12 species of fishes from Upper Rapti River, representing 2 Orders, 4 Families and 9 Genera. The Upper Rapti River provided adequate habitat for *Barilius bendelisis*, *Schizothorax labiatus*, and *Schizothorax plagiostomus* since these were the river's prominent fishes. Cypriniformes, Cyprinidae, and *Barilius* were the dominant order, family, and genus respectively. Summer had the most fish species, while winter had the fewest. The temperature, pH, and dissolved oxygen levels of river water sustain fish life. These variables influence the spatial and temporal variance in fish assemblages. Kruskal-Wallis test provided the p-value of 0.068 among stations and 0.266 among seasons which exceeds 0.05, so that there were no significant difference in the abundance of fish assemblage structure. The RDA revealed that the environmental variables of pH, water temperature, free carbon dioxide and dissolved oxygen were found to be significant variables to shape the fish assemblage. As a result, the current work has attempted to use a number of additional diversity indices to analyze the spatial and temporal variations in fish diversity.

6.2 Recommendations

Following the completion of this research, the following recommendations have been made to local authorities, students, academics, and researchers.

- ❖ The current study solely looks at the spatiotemporal variation of fish assemblages. As a result, in the next years, aspiring students, scholars, and researchers should conduct research on macrophytes, insects, macro invertebrates, and a comprehensive study of fish taxonomy.
- ❖ Natural and human activities such as deforestation, poisoning of river water, bombardment, exploitation of gravel and sand, illicit fishing techniques, and pollution should be prohibited by the local relevant authorities.

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Photo plate I

Photo 1: *Barilius bendelisis*

Photo 2: *Botia almorhae*

Photo 3: *Garra annandalei*

Photo 4: *Garra gotyla gotyla*

Photo 5: *Garra nasuta*

Photo 6: *Glyptothorax telchitta*

Photo 7: *Labeo caeruleus*

Photo 8: *Neolissochilus hexagonolepis*

Photo plate II

Photo 9: *Schistura horai*

Photo 10: *Schizothorax labiatus*

Photo 11: *Schizothorax plagiostomus*

Photo 12: *Tor Mosal*