1. INTRODUCTION

1.1. Background

Spatial and temporal variation of fish assemblages in river occur at scales from micro-habitat to basin and diel to decadal or longer. Knowledge of spatial and temporal variation is valuable for identifying sources of assemblages regulation across the river and interpreting time series data on fish assemblages (Schlosser, 1990). Fish assemblages variation is function of many interconnecting factors, including hydrologic regime, geoclimatic region, species composition, biotic versus abiotic regulation, channel type, disturbance history and frequency (Grossmann *et al.*, 1998). Temporal variation is high in warm water streams and in anthropogenetically disturbed streams. While, spatio-temporal variation of fish assemblages structure depends on channelizes, incise and sand bottom streams (Schlosser, 1982). The river systems are used by freshwater fish as feeding, breeding, nursery site, and migration routes. Fish assemblages in river and lakes display spatial and temporal variation due to anthropogenic activities and environmental fluctuations (Jackson *et al.,* 2001). Human activities, urban and industrial development throughout the world affect the river ecosystem. Eutrophication, river-lake isolation, and over fishing change the fish assemblage structure and diversity (volanthen *et al.*, 2012). Thus fish community are important as a biological indicator of human induced change in river and lake ecosystem (Ru, 2013).

Earth contains abundance of water which covers 71 % of its surface, out of total water bodies about 97 % remain in the seas and remaining 3 % exist as fresh water in lakes, rivers, streams reservoirs, underground water and permanent glacier etc (Wetzel, 1983). However, Nepal is a small mountainous landlocked country comprising of snow-clad Himalayas and has large fresh water bodies which possess 2.27 % of the water resources in the world (DOFD-2013/14), These water bodies support biologically diverse fish fauna and has great potential for hydro power generation (Rai *et al*, 2008; Gubhaju, 2012). The fresh water of Nepal accounts 0.21 % of fish diversity in total global fish diversity and different environmental variables such as temperature, dissolved oxygen, carbon dioxide and minerals are the functional factors to the fresh water life of Nepal (Shrestha, 1995). The river and stream of hilly region dominated by cold water fish species while warm water fish species are dominated in Tarai region. Nepal is rich in fish diversity comprises of 230 species among them 11 exogenous and 16 endemic fish species (Rajbanshi, 2013). Among the recorded 230 species of freshwater fish, approximately 59 species categorized as cold water fish (Petr and Swar et al, 2002) and 21 species are in the IUCN Red List. The fishes of Nepal are very similar to those of Southeast Asia, consisting mainly of carps, catfishes, eels and hill stream fishes (Mishra and Kunwar, 2014).

The fish species are distributed in various water bodies such as rivers, lakes and ponds. The diversity and fish assemblages changes with elevation and seasons. The fish diversity is low in high mountains, moderate in mid hills but high with endemic species and highest in lower foot hills. Animals often shift their diet in response to change in resource availability, abiotic environmental conditions and ontogenetic stage (Stuart *et* *al*., 2004; Werner, 1988). Availability of food resources varies in both space and time, variation in precipitation is another important factor which influencing fish assemblages in tropical streams and rivers. In rainy seasons water bodies expands and forms flood plains due to which habitat of fish expands which support for feeding, spawning and refuge from predators but in dry season habitat shrink results in food resource depletion and higher in consumers densities (Winemiller and Jepseni, 1998). Flood plains are beneficial for fishes and supports in high primary and secondary production and diverse resources (Henderson, 1990).

Studies have shown that the diversity of fish high in monsoon and post monsoon while low in pre monsoon. The species Like *Channa, Puntius, Mystus* are seasonal and highly abundant in specific seasons. It might be due to sufficient food resources and water flow in monsoon and post monsoon while less water flow and high anthropogenetic activities could be cause the less abundance and diversity of fish in pre-monsoon (Oli *et al*., 2013). Nislow *et al*., (2002) and Edds *et al*., (2002) observed lower species richness and abundance in winter season while higher in spring/summer seasons in various lotic water bodies. Similarly Pokharel *et al*., (2011) reported higher species richness and abundance in spring/summer and lower during winter seasons from Seti Gandaki River. Among different environmental variables, the Dissolved oxygen and temperature are most important for fish and highly affect on fish diversity and distribution. The amount of dissolved oxygen and temperature are higher in summer than winter due to which fish diversity is higher in summer (Allan, 1995; Irz *et. al*. 2007).

1.2. Objectives

1.2.1. General objectives

- To investigate the spatial and temporal variation of fish assemblage in Pathariya River of Kailali district.

1.2.2. Specific objectives

- To determine the spatio-temporal variation in species composition and abundance.
- To describe the relationship between certain environmental variables and Fish assemblages structure.

1.3. Research questions

- How does the fish assemblage change along longitudinal and temporal gradient of the River?
- What physical and chemical variables are associated with longitudinal and temporal changes in fish assemblage structure?

1.4. Significance of the study

Recently fishes in Pathariya River have steeply declined due to pollution, harmful fishing practices, habitat modification, environmental degradation and barrier effects of dams and impact of other developmental activities. The physicochemical parameters are also changing in these recent years which are also the cause for the loss in fish diversity. Since this river contributes habitat for wide variety of aquatic species, the status of fish species yet to be known. Therefore, this study will provided information about the variation in fish assemblage of the Pathariya River, which will be the first work in this study area.

2. LITERATURE REVIEW

2.1. Spatial variation of fish assemblage structure

The diversity and distribution of organisms including vertebrates, invertebrates and plants correlated with altitude. Generally species diversity decrease with increase in altitude (Lomolino, 2001). There are different factors which causes the reduction of diversity in high altitude. Some of them are decrease in primary productivity with increase in altitude, reduction of suitable available area for organism, unsuitable climatic condition and reduction in the food resources (Hutson, 1994). The factor which determine the fish assemblages structure in riverine of both temperate and tropical regions are altitude, river size, temperature, water velocity, depth, habitat complexity (Tejerina-Garro *et al.,* 2005). The diversity and fish assemblages structure are correlated with elevation and the distance from the origin of water body. Many previous studies have shown that fish species richness is in high in lowland and low species richness in highland. In Columbia the diversity of mountain fishes are highest in the northern Andes region including 37 % of endemic fish of 220 species and in every 1000 m increase in altitude 19.7 species were found to be decreased (Rodriguez *et al.,* 2016, Jaramillo *et al.,* 2010). Several studies have reported a monotonic decrease in fish species richness with increasing elevation (Jaramillo *et al.,* 2010 and Juna *et al.,* 2015). Many factors such as climatic, spatial, and biotic have been suggested as underlying causes of elevation patterns of fish species distribution. The dynamics of water flow bodies determined by the climate and topography (Allen, 1995). In the mountain latitudinal gradient, the water of river and stream is cold, turbulent and highly oxygenated in the highest reaches while in the lowest reaches the water is warmer, less turbulent and oxygen amount is less. Due to these changes structure greatly affect on diversity and fish assemblages according to the altitudinal variation (Joccobsen, 2008).

Nepal is a second richest country in the world in fresh water resources. Its elevation ranges from 59 m to 8848 m and geographically divide into three regions. The Tarai region is warm with low altitude and plain lands, hilly region has moderate climatic condition with low mountains and Himalayan region has cold climatic condition with high mountains. Due to these climatic conditions and altitude each region has particular species of fishes. In Nepal the diversity and species richness were also decreased with increased in elevation (Shrestha, 2008). The warm water fish species such as *Labeo rohita, Catla catla, Cirrhinus mrigala, Cyprinus carpio, Cytnopharyngodon idellus* are important cultivated fish species are highly dominated in Tarai region (DOFD, 2006/07). The cold water fish species such as *Neolissocheilus hexagonolepis, Schizothoraichthys spp, Schizothorax spp,* and *Tor sp*p are most economically important and are dominated in hilly streams (Shrestha, 1981). The endemic fish species of Nepal like *Schizothorax macropthalmus*, *S. nepalensis, S. raraensis* are found in highest elevation about 3200 m in Rara lake (Shrestha, 2008).

2.2. Temporal variation of fish assemblage structure

The temperature is one of the most important factor limiting the fish diversity in the tropical and great altitude (Jacobsen, 2008). All aquatic organisms like fish, insects, zoo plankton, and phytoplankton have different temperature ranges. The water temperature greatly influences on water chemistry and high water temperature cause the fluctuation in DO and pH. Except that water temperature influence on aquatic life cycle, metabolism and behaviour of fish and high water temperature leads to the thermal stratification in the river and lake which may lead reduction in diversity and unequal distribution of fish (Jain *et al*., 2013). The temperature decrease with increase in altitude. The daily fluctuation of temperature is high at highland area in comparison to low land area (Bussion *et al*., 2008). The physicochemical parameters including temperature of water greatly effect on fish assemblages so that fish assemblages varied according to temperature variation. The fish assemblages have used as ecological indicators to evaluate health of water as well as level of degradation (Basavaraja *et al*., 2014). The fish assemblages structure correlated with environmental factors, stream size, water flow and available nutrients (Negi and Mamgain, 2013). The physicochemical parameters like pH and DO are lower at the lower streams and higher at the higher streams while the CO_2, nitrogen and phosphorus compounds are higher at the lower streams and lower at the higher streams (Kannel *et al*., 2008). The physicochemical and microbiological characteristics of water described the quality of water. According to Bhandari and Nayal (2008) the physicochemical variables such as chloride with pH, Mg, Na, hardness, total suspended solid are positively correlated while the negative correlation was found with potassium, chloride, hardness and turbidity. The fish assemblages structure more or less correlated with different environmental variables. In Seti Gandaki river, the fish assemblages structure is positively correlated with conductivity, CO_2, phosphate and nitrogen compounds while the negatively correlated with pH and DO (Pokhrel, *et al*., 2018).

The fish communities and physiochemical parameters including temperature in rivers and lakes are fluctuate according to seasons (mehner *et al.,* 2005). The fish assemblages are typically dynamic, reflecting, the changing suit of environmental conditions to which they are exposed on short term or seasonal bases (Termain and Adams, 1995). The fish is cold blooded animals, according to environment its body temperature changes and high fluctuation of water temperature affect on fish health (Bhatnagar*et al.,* 2013). The global level of temperature for tropical fishes is 28 °C to 32 °C,for cold water fishes less than 12 °C is suitable and greater than 35 °C is lethal to the maximum number of fishes (Bhatnagar *et al*., 2004) while for the carp culture 24 °C to 30 °C temperature is suitable (Santosh and Singh, 2007). The fish like *Labeo, Mystus, Puntius, Channa* are warm water fish species which are dominated in the river of warm water and the *Labeo, Catla, cirrhinus* and Carp are major fish species which are used for aquaculture in Tarai region of Nepal because these species can survive in high temperature (Rai, *et al*., 2008). While the *Schizothorax, Gara, Glypothorax, Pseudocheneis* fish species are dominated in cold water river of hilly region, these fish species have low capacity to resist in high temperature (Koel *et al*., 2003).

Despite a number of studies related with aquatic diversity from different parts of Nepal, there is still no study has been conducted on the fish assemblage structure in Pathariya River. Therefore, this study is designed to investigate the spatio-temporal variation in fish assemblage structure of Pathariya River, Western Nepal.

3. MATERIALS AND METHOD

3.1. Study area

Pathariya River is located in Kailali district Western, Nepal, having the total length of 45km. The study was carried out from Malbhanga to Dhunganatol covering the length of 36km. The Pathariya River originates from Churia hill on north and finally discharges into Mohana River at Dhunganatol on south. It lies between 28º22' and 29º05' North latitude and 80º30'to 81º18' East longitude. Altitude ranges 109 m to 1950 m from sea level, climate varies from tropical to sub tropical, average rainfall is 1840mm, average annual temperature in Autumn reaches maximum 43°C and minimum 24°C while in winter maximum temperature is 19°C and minimum temperature is 15°C.

Four station (A, B, C and D) for study were selected representing upstream, urban and downstream sites from Malbhanga to Dhunganatol based on accessibility/human disturbances, altitudinal variation, dams and confluences meeting of other tributaries (Figure 1).

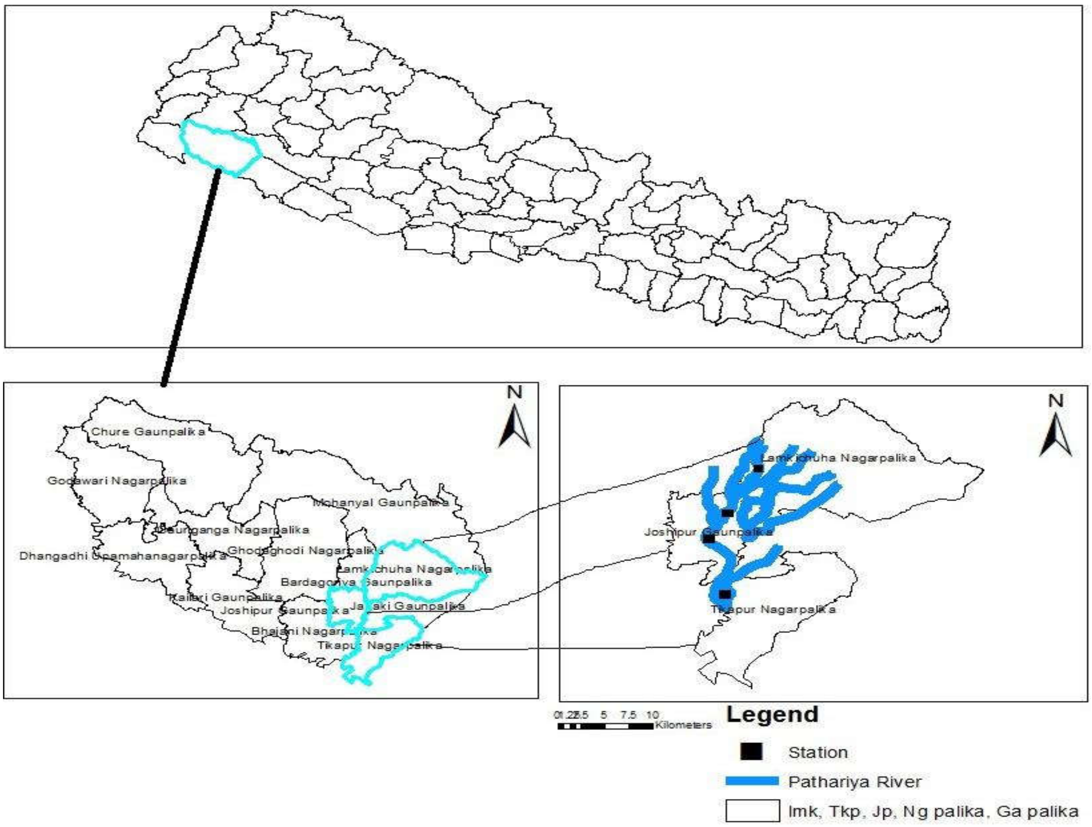

Figure 1. Map showing the study area

Station A

The station A lies at the Malbhanga of Lamki Chuha Mucipality and it is about 5 km far from Mahindra highway at Gulra towards North. The boulders, large amount of pebbles, gravels and sand with medium velocity of water were found in this station. Mostly gravels and sand were found in river bed. Vegetation is dominated by Bamboo on bank of the river and human settlement is low on the station.

Station B

The station B was selected at the Thakurdwara of Joshipur Rural Municipalitywhich is about 10 Km South from Mahindra highway at Gulra. At this station irrigation dam was found which was built on 2028 BS and supply water to 2000 ha for irrigation. The plain agricultural lands were found on the both side of bank of the river. River bed was formed by gravels and sand and human settlement is higher in this area than the station A.

Station C

The sampling station C was situated at Sonalipur of Joshipur Rural Municapalitywhich is approximately 6 km far from station B to Southwards. Another irrigation dam was also found in the Pathariya River at this station which was built on 2046 BS and supplies the water to 2500 ha for irrigation. There was also agricultural land on both side of bank of the river.

Station D

The sampling station D was located on the Dhunganatol of Tikapur Muncipality which is about 15 Km downstream of station C. At this station Pathariya River finally mix in the Mohana River. Water velocity is higher than all other station. River bed was mostly formed by sand. There were Agricultural land and human settlements on both sides of the bank of river.

3.2. Sampling

For the present investigation the field work was conducted from September 2017 to August 2018 with four seasons- Autumn (September, October and November), Winter (December, January and February), Spring (March, April and May) and summer (June, July and August). Fishes were sampled at 4 sites using a medium size cast net of mesh size ranging from 1.5 cm to 2.5 cm and Gill net having 2-3 cm mesh size, 30-35 feet length and 3-4 feet width, with the help of local fisher man. These fishing gears were operated within 100 m area of each site for 1 hour in each station at 9 AM. Total 40 throws were made for cast net and 4 hauls for gill net to catch fishes. For estimation of abundance of fishes, two pass removal method (Seber and Le cren, 1967) was used. Each removal pass include moving first upstream/river then downstream/river within a pre-determined length (100m) with equal effort 30 minutes for each pass at each site of the river

3.3. Identification of specimen

The collected fishes were counted, examined and identified based on their key morphological characters. Fish samples that seemed difficult to identify on spot were preserved in 10% buffered formalin and brought to Central Department of Zoology, Tribhuvan University for further study. Finally, identification of fish was carried out following the taxonomic keys of Shrestha (1981, 2001), Talwar and Jhingran (1991), Jayram (1999), and Shrestha (2008).

3.4. Physical analysis of water

Water samples were collected and different physical properties were analyzed following the standard methods of Adoni (1985), Trivedy and Goel (1984), and American Public Health Association (APHA, 1998).

Water velocity

The river's velocity was measured by the simple method of timing a float with stopwatch. The float material was squeezed lemon which was tied with a rope and left in the river and the time to cross the point was calculated and this velocity was expressed in m/s.

Temperature

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

Transparency

The transparency of the water was measured by Sechii disc method. The metallic plate of 20cm diameter with four alternatively black and white quadrants on the upper surface and a hook at the middle to tie a rope was used. This disc was dipped in the water and the depth was noted at which it just disappeared. Then conversely, it was raised gradually to note the depth at which reappeared and the calculation was done using following formula.

$$\text{Transparency} = \frac{\text{Just appearance} + \text{Just disappearance}}{2}$$

Turbidity

Turbidity of the water is inversely proportional to the transparency. Hence, the turbidity of the river water was calculated by using transparency value into the following equation.

$$\text{Turbidity (x)} = \frac{1000}{1.568Y - 1.2752}$$

Where, X = turbidity

Y = transparency

3.5. Chemical analysis of water

The chemical parameters were analyzed after (APHA 1998), (Adoni 1985) and (Trivedy and Goel 1986).

Hydrogen ion concentration

A pH meter was used to record the hydrogen ion concentration of water during the study period at every station of the Pathariya River.

Dissolved Oxygen

The dissolved oxygen of water was calculated using Winkler's method. The sample of water from every station was collected in a BOD bottle without bubbling. Two milliliters of manganese sulphate and similar quantity of alkaline- iodide- azide solution were added and shaken. Brown precipitation was obtained which was again dissolved by adding 2ml of conc. Sulphuric acid. Then this solution was titrated against standard sodium thiosulphate solution (0.025N) and the calculation was carried out using formula

$$\text{DO (mg/l)} = \frac{ml \times normality\ \ of\ titrant\ \times 8 \times 1000}{V_2\{(V_1 - V)/V_1\}}$$

Where, V = Volume of $MnSO_4$ and KI added

V_1= Volume of BOD bottle

V_2= Volume of the part of the content titrated.

Free Carbon dioxide

To determine the free CO_2, 50 ml of sample water was taken and few drops of phenolphthalein indicator were added. Thus obtained colourless solution indicated the availability of carbondioxide. Now this solution was titrated against standard alkali titrant (Sodium hydroxide 0.02272 N) to the slight pink end point. Free carbondioxide in the water sample was calculated using formula

$$\text{Free } CO2 = \frac{(\text{ml} \times \text{N}) \text{ of NaOH} \times 1000 \times 44}{V}$$

Where, V = Volume of water sample taken (ml)

Hardness

It was measured by EDTA titrimetric method.

$$\text{Total hardness as } CaCO_3 \text{ (mg/l)} = \frac{mlofEDTA \times 1000}{mlofsample}$$

3.6. Statistical Analysis

Species Diversity Index

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

Shannon Weiner diversity index is designated as H', which is calculated as:

$H' = -\Sigma (ni /N) \log (ni/N)$

Or, if $Pi = ni/N$

$H' = -\Sigma Pi \log_e Pi$

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.

Pi= ni/ N = Relative abundance of each species, calculated as the proportion of individuals of a given species to the total number of individuals in the community.

Species richness index (d)

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

Margalef species richness (d) = S-1/ log N

Where, S= Number of species

N = Number of individuals

Evenness index

To calculate whether species are distributed evenly across seasons and across landscapes elements, evenness index was determined by the following equation (Pieleu 1966).

E= H'/ log 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.

Multivariate analysis

The relation between species diversity and environmental variables were analyzed by redundancy analysis (RDA) method (Braak,and Cajo 1988) by using vegan library in 'R' (Oksanen et al. 2019).

4. RESULTS

4.1. Spatio-temporal variation of Fish assemblage structure

4.1.1. Systematic position of fishes

Pathariya River was found to be enriched by various types of fishes. Total of 407 individuals of fishes were collected from four stations during the four different seasons. These fishes were classified according to Shrestha (1981), Jayaram (1999), and Shrestha (2008) into 25 fish species belonging to 4 orders, 8 families and 17 genera. Systematic position of fishes shown in table 1.

Table 1: Systematic position of fishes

S.N.	Order	Family	Genus	Species
1	Cypriniformes	Cyprinidae	*Puntius*	*Puntius sophore*
				Puntius terio
				Puntius ticto
			Labeo	*Labeo rohita*
				Labeo calbasu
				Labeo caeruleus
			Cirrhinus	*Cirrhinus mrigala*
				Cirrhinus reba
			Barilius	*Barilius barila*
			Aspidoparia	*Aspidoparia morar*
			Esomus	*Esomus danricus*
			Cytnopharyngodon	*Cytnopharyngodon idellus*
		Cobitidae	*Acanthocobotis*	*Acanthocobotis botia*
			Lepidocephallus	*Lepidocephallus guntea*
				Lepidocephallus menoni
2	Siluriformes	Bagridae	*Mystus*	*Mystus tengra*
				Mystus bleekeri
				Mystus vittatus
			Aorichthys	*Aorichthys seenhala*
		Claridae	*Clarias*	*Clarias batrachus*
		Siluridae	*Ompok*	*Ompok bimaculatus*
3	Perciformes	Channidae	*Channa*	*Channa punctatus*
		Nandidae	*Nandus*	*Nandus nandus*
4	Synbranchiforms	Mastacembelidae	*Macrognathus*	*Macrognathus aral*
			Mastacembelus	*Mastacembelus armatus*

4.1.2. Fish distribution and frequency occurrence in the Pathariya River

During the study period, a total 25 species of fish were recorded. *Puntius ticto* was dominant and *Labeo caeruleus* was least abundant species. All the fishes recorded in all the sampling stations and their frequency distribution are given in table 2.

Table 2: Distribution and frequency of fishes

S.N.	Scientific name	Local name	Sampling station											
			Station A				Station B				Station C			
			Sep	Dec	Mar	July	Sep	Dec	Mar	July	Sep	Dec	Mar	July
1	*Puntius sophore* (H. buchanoan) 1822	Pate sidhra	5	2	-	3	4	2	-	6	4	1	-	2
2	*Puntius terio* (H. buchanoan) 1822	Pothi	-	-	-	-	3	-	3	2	2	-	1	-
3	*Puntius ticto* (H. buchanoan) 1822	Tite pothi	7	4	3	-	5	4	1	-	5	2	1	2
4	*Labeo rohita* (H. buchanan) 1822	Rohu		-	-	-	-	-	-	-	2	-	-	-
5	*Labeo calbasu* (H. buchanan) 1822	Gerdi	-	-	-	-	-	-	-	-	-	-	-	-
6	*Labeo caeruleus* (Day) 1878	Bishari	-	-	-	-	-	-	-	-	-	-	-	-
7	*Cirrhinus mrigala* (H. buchanan) 1822	Naini	-	-	-	-	2	-	2	2	3	1	2	-
8	*Cirrhinus reba* (H. buchanan) 1822	Mrigal	-	-	-	-	-	-	-	-	-	-	-	-
9	*Barilius barila* (H. buchanan) 1822	Faketa	6	2	2	3	-	-	-	-	-	-	-	-
10	*Aspidoparia morar* (H. buchanan) 1822	Chakale	-	-	-	-	5	2	1	3	3	2	-	1
11	*Esomus danricus* (H. buchanan) 1822	Dedhawa	3	-	2	3	2	2	3	1	4	3	2	-
12	*Cytnopharyngodon idellus*(valenciennes)	Grass carp	-	-	-	-	-	-	-	-	-	-	-	-
13	*Acanthocobotis botia* (H. buchanan) 1822	Baghe	5	1	-	-	-	-	-	-	-	-	-	-
14	*Lepidocephalus guntea* (H. buchanan) 1822	Kande	4	-	3	-	-	-	-	-	-	-	-	-
15	*Lepidocephalus menoni* (Pillai andYazdani) 1976	Goira	-	-	-	-	-	-	-	-	-	-	-	-
16	*Ompok bimaculatus* (Bloach) 1797	Nauni	-	-	-	-	-	-	-	-	-	-	-	-
17	*Mystus tengra* (H. buchanan) 1822	Tenger	4	2	-	5	3	2	-	1	4	-	-	3
18	*Mystus bleekeri* (Day) 1878	Temger	-	-	-	-	-	-	-	-	-	-	-	-
19	*Mystus vittatus* (Bloach)1797	Tenger	-	-	-	-	2	3	-	1	5	2	-	-

20	*Aorichthys seenghala* (H. buchanan) 1822	Sujaha	-	-	-	-	-	-	-	-	3	-	1	-
21	*Clarias batrachus* (Linnaeus) 1768	Mangri	-	-	-	-	-	-	-	-	2	-	-	-
22	*Channa punctatus* (Bloach) 1793	Helae	4	-	2	1	2	1	-	3	3	2	1	1
23	*Nandus nandus* (H. buchanan) 1822	Dhoke	-	-	-	-	-	-	-	-	2	-	-	-
24	*Macrognathus aral* (Blaoch and Schneider) 1801	Bami	5	1	3	3	4	-	1	-	2	-	1	1
25	*Mastacembelus armatus* (Lacepede) 1800	Bami	-	-	-	-	-	-	-	-	3	1	-	1
	Total													

4.1.3. Order wise frequency occurrence of fish in Pathariya River

The collected fish were classified into four orders i.e. cypriniformes, siluriformes, perciformes, and symbranchiformes. Cypriniformes was the most dominant order constituting 59.21 % of the total fish population followed by Siluriformes (19.91%), Synbranchiformes (11.3%) and Perciformes (9.58%) (Figure 2).

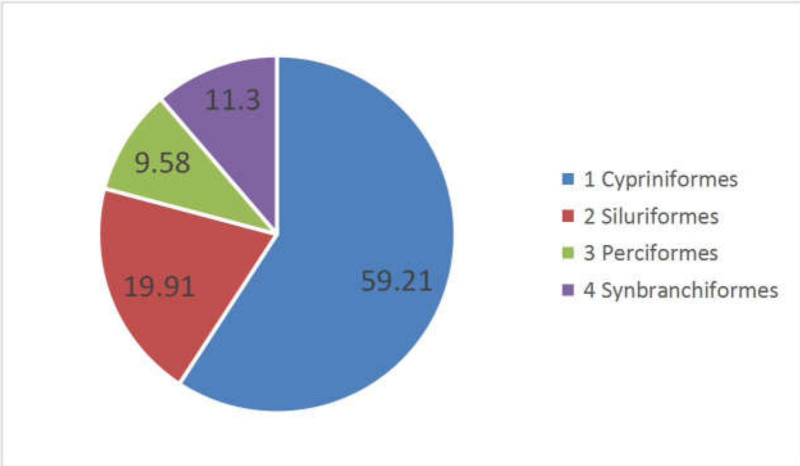

Figure 2: Order wise frequency occurrence of fish

4.1.4. Family wise occurrence of fish in Pathariya River

The study recorded Nine families of fish species in which Cyprinidae family was most abundant (53.56%) followed by Bagridae (17.44%), Mastacembelidae (11.31%), Channidae (8.11%), Cobitidae (5.65%), Claridae (1.72%), Nandidae (1.47) and Siluridae (0.74%) (Figure 3)

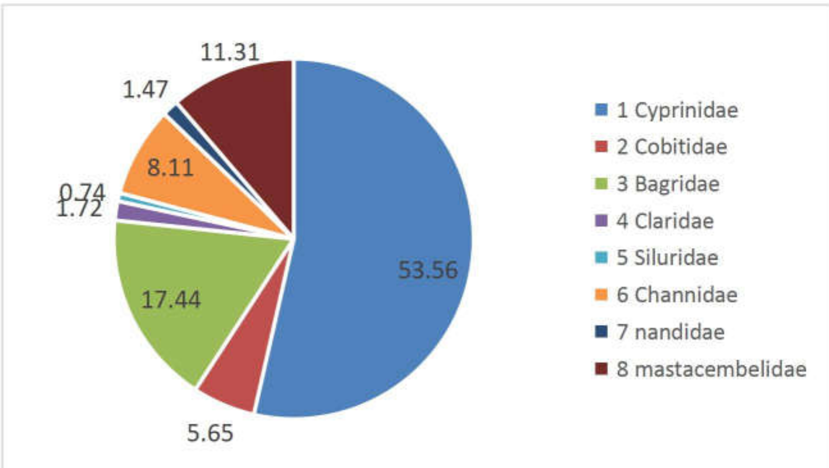

Figure 3: Family wise frequency occurrence of fish

4.1.5. Diversity indices

The value of Shannon Wiener diversity index and Margaleaf richness were calculated according to month and stations. Highest Shanon weiner diversity index was found in station D (2.96) and the lowest was found in station A (1.67). Similarly, higher Shannon diversity index values were found in September where low during March. The value of Shannon-weiner diversity in different station and in different seasons are presented in figures 4 and 5. The maximum Margalef richness value was observed 22 at station D where minimum value was observed 6 at station A. Higher Margalef richness value was found during September where lower value was observed during March. The value of Margalef richness in different station and in different seasons are presented in figures 6 and 7.

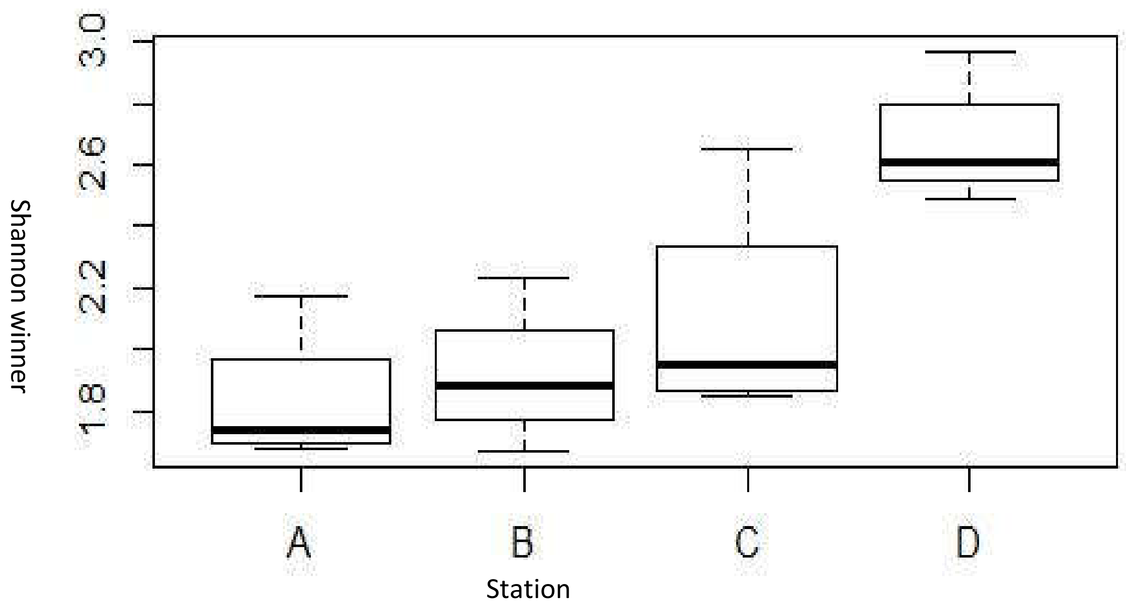

Figure 4: Relationship between station and Shannon winner diversity index of fish of Pathariya River

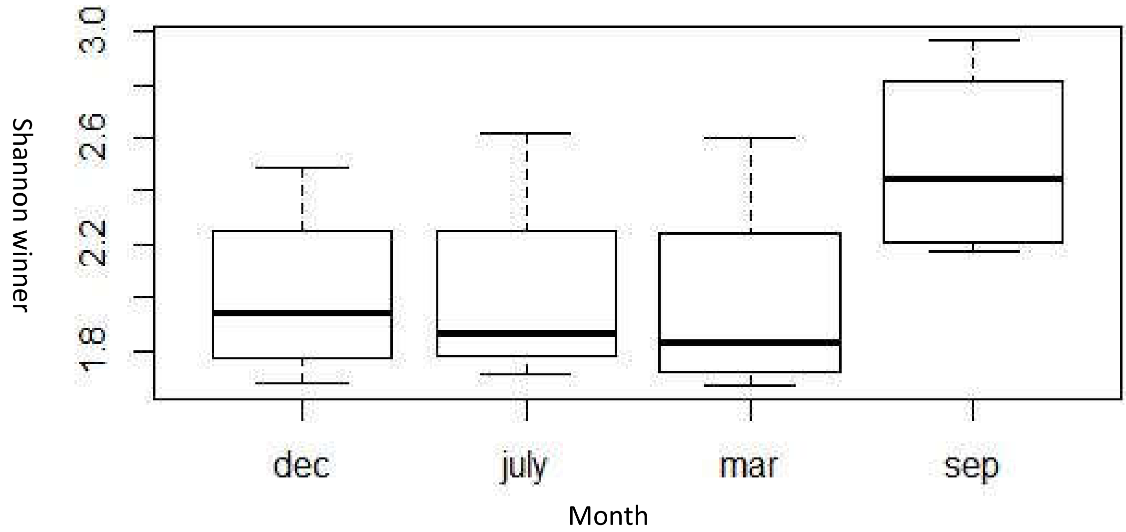

Figure 5: Relationship between month and Shannon winner diversity index of Pathariya River

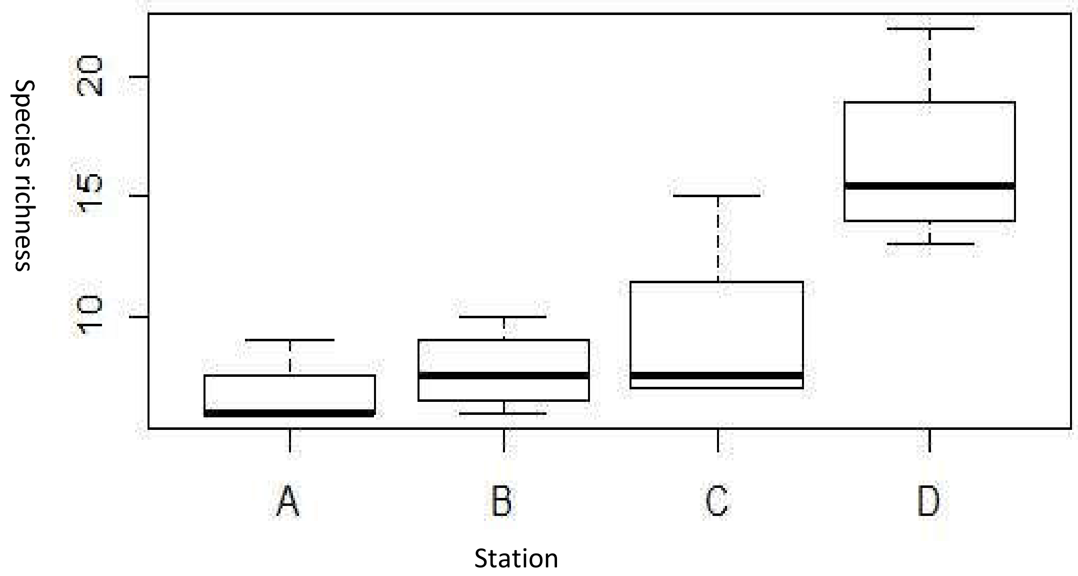

Figure 6: Relationship between station and species richness of fish in Pathariya River

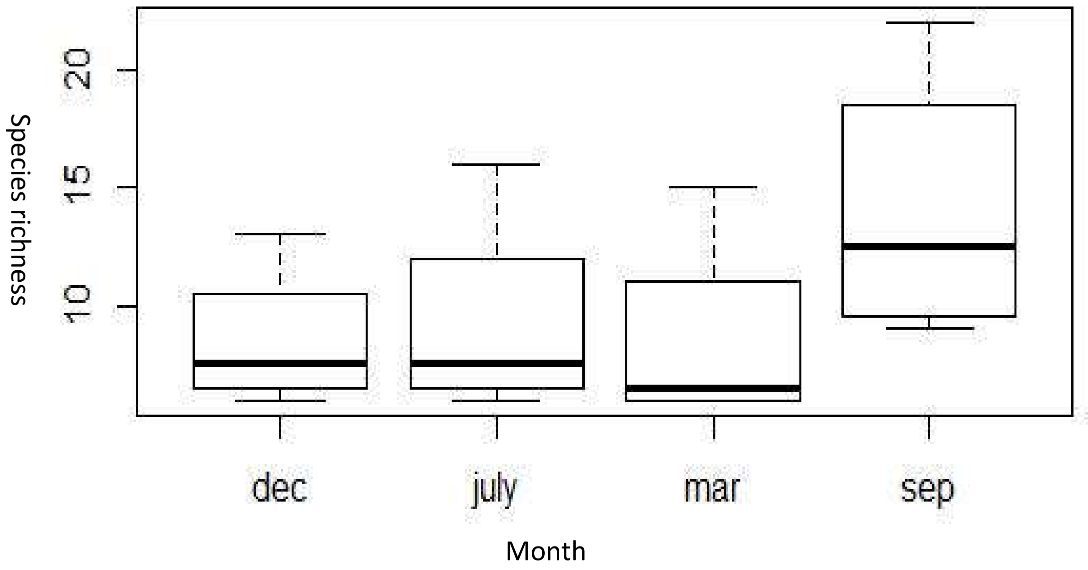

Figure 7: Relationship between month and species richness of fish in Pathariya River

4.2. Spatial and temporal variation of the environmental variables

Temperature

Temperature plays vital role in distribution of fishes in river. The highest temperature (29°C) was recorded at stations C and D in March. The lowest temperature (15°C) was recorded in station D in December. The variation of temperature at different stations in different month of study period is given in figure 8.

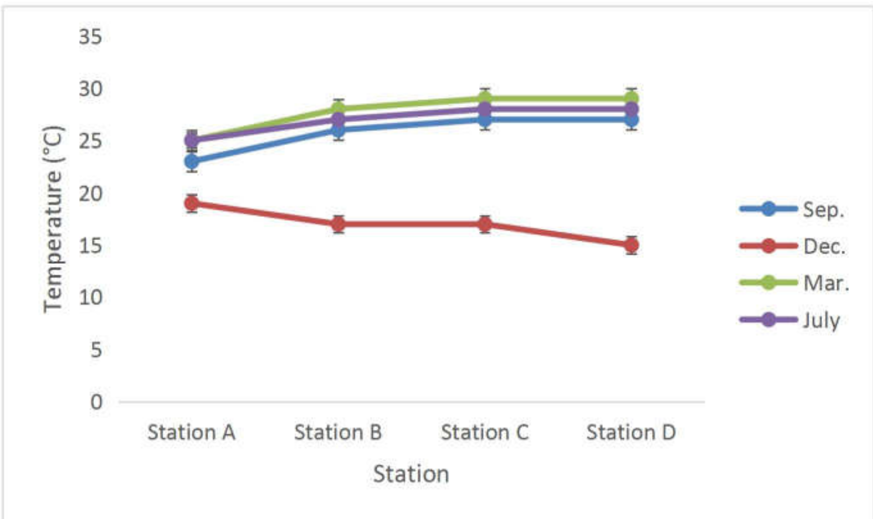

Figure 8: Seasonal Variation of water temperature in different stations

Transparency

During the study period both the highest (77cm) and lowest (7cm) transparency were seen at station D. Water transparency is high during the month of March and low in July. The level of transparency at different stations in different month is given in the figure 9.

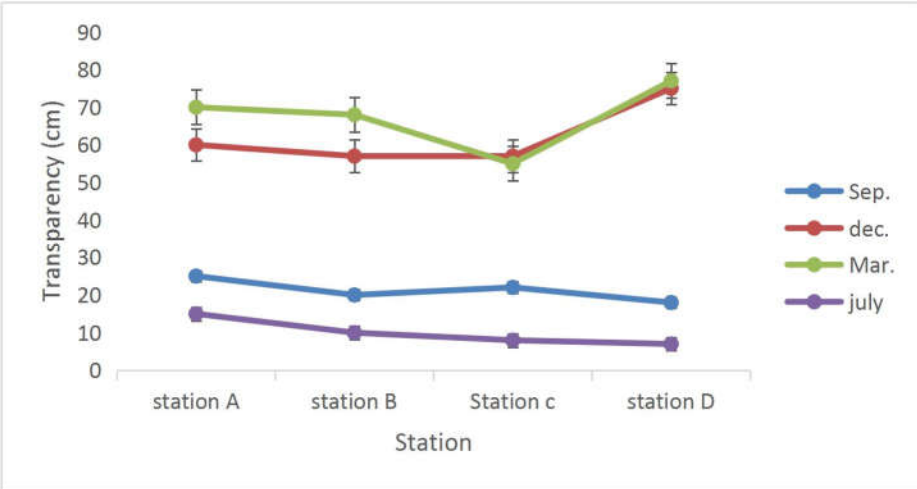

Figure 9: Variations of water transparency (cm) in different stations

Velocity

Velocity of river greatly affects the distribution of fish species. During the study period, the velocity of Pathariya River ranged minimum from 0.03 m/s at station C in the month of December to maximum of 2.16 m/s at station D in the month of July. The variation in velocity of River water in different station is given in the Figure 10.

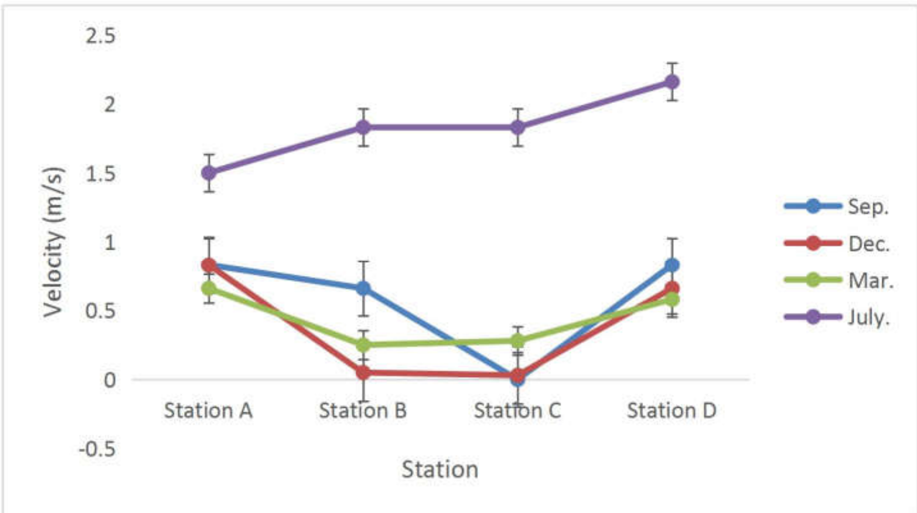

Figure 10: Variation in water velocity (m/s) at different stations

Hydrogen ion concentration (pH)

pH is the measurement of the negative logarithm of hydrogen ions concentration, which is greatly influenced by the concentration of CO_2 temperature of water. The pH of Pathariya River remains slightly alkaline at all stations throughout year. Both the highest (8.4) and lowest value (7.5) of pH were recorded in station D during the months of March and July respectively. The variation in pH of River water in different station is given in the Figure 11.

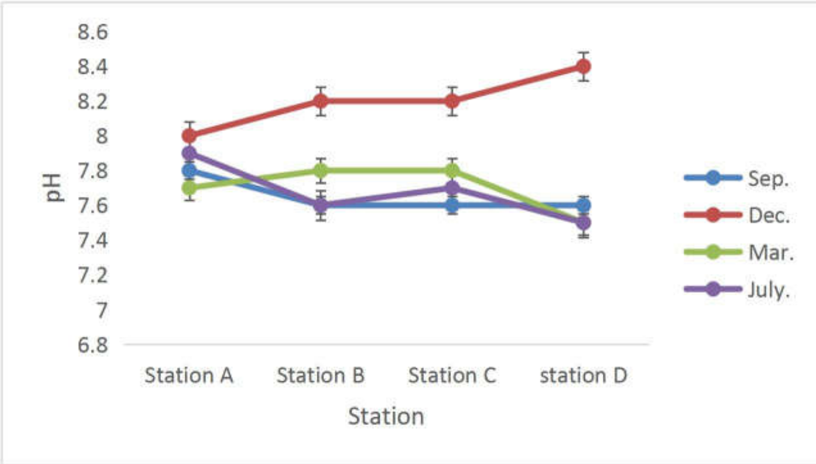

Figure 11: Variation in pH value at different stations

Dissolved oxygen (DO)

The main source of oxygen in water is photosynthetic plankton and atmospheric air. The concentration of DO was highest (10.66mg/l) in stations A and D during the month of July. While the lowest concentration of Do (6.56mg/l) was seen at the station C in December. The variation in DO of River in different station is given in (Figure12).

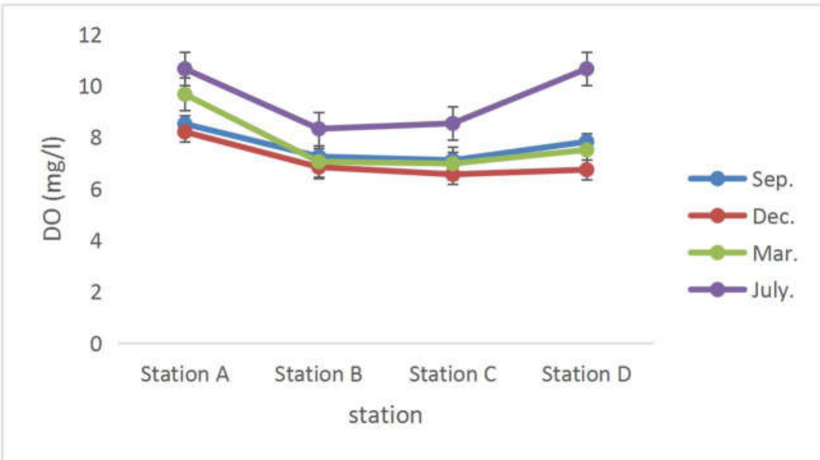

Figure 12: Variation in Dissolved Oxygen (mg/l) at different stations

Free carbondioxide (CO_2)

The highest calculated value of free carbondioxide was foind to be 11.85 mg/l in station C in December. Free carbondioxide value was detected to be lowest (6.32 mg/l) in station D in March (Figure13).

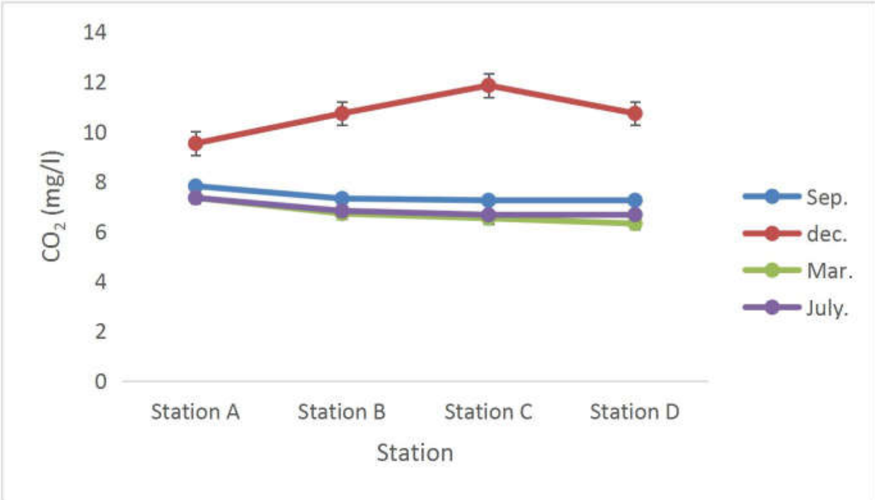

Figure 13: Variation in free carbon dioxide (mg/l) at different stations.

Hardness:

The hardness of the water ranged from 150 mg/l to170 mg/l. The hardness of the water was found to be highest at site D in summer season (July) and lowest hardness was found to in station A in March (Figure14).

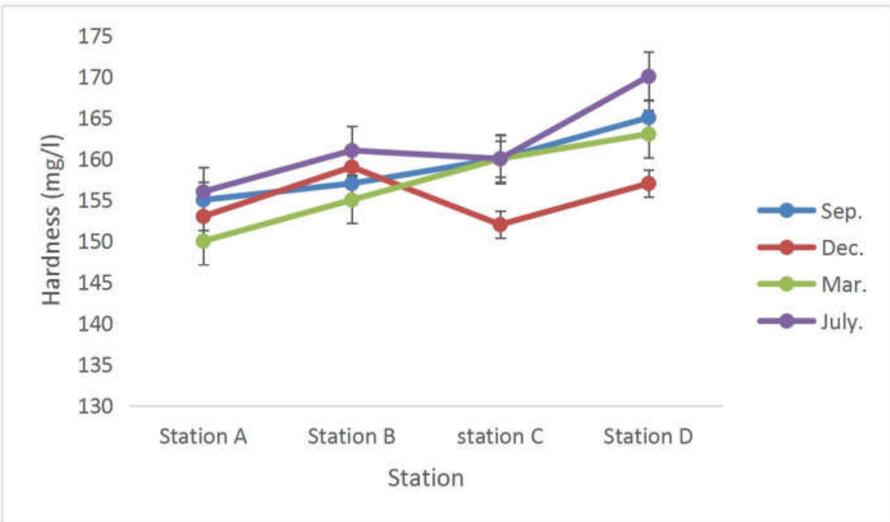

Figure 14: Variation of Total Hardness at different sampling sites and seasons

4.3. **Relationship between fish assemblage and environmental variables**

To determine the relationship between the environmental variables and fish assemblages structure the multivariate analysis was used. At first data was entered in R- software then run for DCA and obtained the axis length and eigenvalues. The axis length was 2.32 and eigenvalues was 0.36 (table 3). So that to identify the relationship between the environmental variables and fish assemblages structure, Redundancy analysis (RDA) was performed.

Table 3: data for RDA analysis

	DCA1	DCA2	DCA3	DCA4
Eigenvalues	0.36	0.13	0.08	0.07
Decorana values	0.39	0.09	0.06	0.02
Axis length	2.32	1.94	1.19	1.29

There was significant correlation between certain environmental variables and fish abundance while some environmental variables are none significantly correlated with fish abundance. The species richness and hardness, Shannon winner diversity index and hardness were significantly correlated, while species richness and Shannon winner diversity index were none significantly correlated with temperature, transparency, velocity, pH, DO and CO_2. The correlations between the environmental variables such as transparency and velocity, transparency and pH, velocity and DO, velocity and hardness, temperature and pH, hardness and pH were significantly correlated. The value of correlation (r) and level of significance (p) between different variables were shown in appendices 1.

The correlation of abundance of individual fish species towards different environmental variables were different and the positioning of total 25 captured fish species in relation to environmental variables is shown in figure 15. The cyprinids like *Esomus danricus, Barilius barila, Puntius sophore, Puntius ticto* preferred positively correlated with DO, pH, CO_2, transparency and negatively correlated with velocity and temperature. Likewise several cyprinids like *Puntius terio, Cirrhinus mrigala, Cirrhinus reba, Aspidoparia morar, Cytnopharyngodon idillus, Labeo rohita, Labeo calbasu, Labeo caeruleus*, favoured positively correlated values of velocity and temperature while negatively correlated with DO, pH, CO_2 and transparency. Among cobitidae *Lepidocephalus guntea, Acanthocobotis botia* favoured positively correlated values of DO and negatively correlated with velocity and temperature while among cobitidae *Lepidocephalus menoni* favoured positively correlated with temperature, velocity while negatively correlated with pH, DO, CO_2 and transparency. Among bagridae *Mystus tengra* positively correlated with DO and negatively correlated with temperature and velocity while among bagridae *mystus vittatus, Mystus bleekari* fvoured positively correlated with velocity and temperature while negatively correlated with pH, CO_2, transparency and DO. Among claridae, siluridae, and Nandidae the *Clarius batrachus, Ompok bimaculatus and Nandus nandus* respectively favoured positively correlated with velocity and temperature while negatively correlated with DO, pH, CO_2, and transparency. Among channidae the *Channa punctatus* was more or less positively correlated with DO while negatively correlated with transparency. Among mastacembelidae the *Mastacembelus armatus* was positively correlated with velocity and temperature and negatively correlated with DO, pH and transparency. While *Macrognathus aral* is positively correlated with DO and negatively correlated with velocity and temperature.

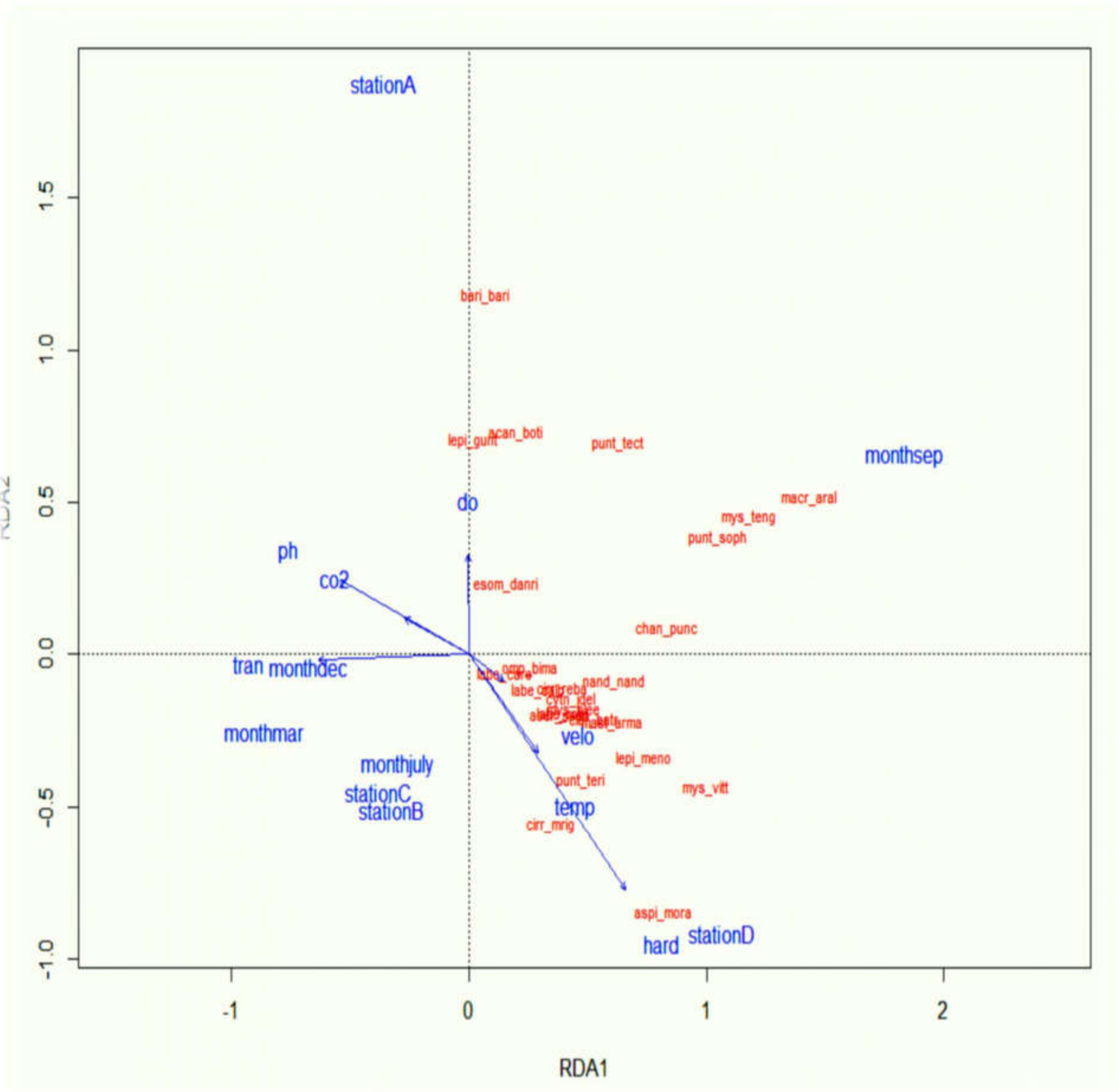

Figure 15: RDA ordination of fish species in relation to sites and environmental variables in Pathariya River

5. DISCUSSION

5.1. Spatio-temporal variation of fish assemblage structure

In the present study a total of 25 species of fish were recorded belonging to 4 orders and 8 families. Among four orders Cypriniformes was the largest order which was followed by Siluriformes, Symbranchiformes and perciformes was smallest order. Among 8 families Cyprinidae include higher number of fish species followed by Bagridae, Mastacembelidae, Channidae, Claridae, Nandidae, and smallest family was Siluridae which comprised least number of fish species. Among 25 fish species *Puntius ticto* was recorded in largest number and followed by *Puntius sophore* and *Mystus tengra* while *Labeo caeruleus* was recorded in smallest number and followed by *Labeo rohita*, *Ompok bimaculatus*, and *Cytnophyrngodon idelius*. Similar to this study Yadav, (2017) reported that cyprinidae was the largest family followed by Channidae, Mastacembelidae, Cobitidae, Netopteridae, Bagridae, Claridae, Nandidae and Belonidae from Bagmati River Similarly Shrestha *et al* (2009) from Tamor River, Saund *et. al.* (2012) and Gautam *et. al*. (2016) from Mahakali River, and Joshi *et al* (2017) from Ghodaghodi Lake also found that cyprinidae as a common fish family in their respective study areas. In the present study the abundance of fish was found to low in winter and high in summer. Pokharel, (2011) from Seti Gandaki River, Jaramilla-Villa *et. al*. (2011) from central Andes Columbia, Jaun, *et. al*. (2015) from northern Anedes Columbia reported higher fish abundance in summer and lower in winter. This is due to change in environmental variables and these variables are mostly influences by altitude and seasons. Some species are seasonal like *Puntius, Mystus* and *Channa* are highly abundant in Pathariya River in summer season and their abundance decrease in winter season. Similar type of results was also found by Oli *et. al*. (2013) from Rampur Ghol, and Rizal, (2015) from Tinau River. This may be due to availability of food resources, habitat area, environmental factors, and refuge from predators.

5.2. Spatial and temporal variation of the environmental variables

The physiochemical parameters of water like temperature, transparency, velocity, pH, DO, CO_2 and hardness play vital role in abundance and species richness and these parameters are greatly affected by seasons and elevations (Pokharel *et al*. 2018). In present study the highest temperature and transparency was observed in spring, lowest temperature and transparency was observed in winter and summer seasons respectively. Yadav (2017) also found lower temperature during winter in Bagmati River. The variation of temperature could be due to seasons and altitude. The fish abundance and species richness are positively correlated with water temperature is an important factor which affect on growth and development of fishes. Santosh and Singh (2007) suggested that transparency between 30cm to 40cm is suitable for high productivity of fish pond. The different factors like dispersion of plankton, suspended clay particles, organic matter, pigment as well as human activities also affect on transparency of the river water.

Generally velocity of water decreases in downstream but in the present study velocity of water found to be increased at the downstream (station D). It could be due to the impacts of tributary or mainstream. The chemical parameter of water such as (pH) greatly influence for the survival of fish in both lotic as well as lentic water system. In the present study the water of Pathariya River was slightly alkaline. Rijal (2015) from Tinau River and Yadav, (2017) from Bagmati River also reported slightly alkaline water. According to Santosh and Singh (2007) the suitable value of pH for fresh water fish species ranges from 7 to 8.5 above and below of this is stress full for fish. The value of pH greatly influence by concentration of CO_2 gas. Both high and low level of DO is fatal to the fish species. DO is the important factor which affects the distribution, growth, survival, physiological and behavior of fishes. In the study period the highest value of DO in summer and lowest value of DO in winter were recorded. The amount of required dissolved oxygen is different for different fish species and it depends on seasons and weathers. Bhatnagar *et al. (*2004) reported that suitable amount of oxygen level is greater than 5 mg/l for fish. While according to Santosh and Singh (2007) cat fish and other air breathing fishes can survive in low concentration of oxygen less than 4 mg/l. The solubility of oxygen in water decreases due to increase in salinity, temperature, low atmospheric pressure, high amount of plankton and submerged plants in water. Free carbondixide is highly soluble in water and the main sources of CO_2 in water from atmospheric CO_2 and respiration of aquatic animals. The high concentration of CO_2 causes the reduction in concentration of pH. In the present study, the highest amount of CO_2 was observed in winter (December) while the lowest in spring (March) seasons. According to Boyd and Lichtkoppler (1998) fish avoid free CO_2 levels as low as 5 mg/l but most species can survive in water containing up to 60 mg/l in running water. However, according to Santosh and Singh (2007) the CO_2 in water less than 5 mg/l support good fish production in pond. Hardness is the measure of alkaline earth metals such as calcium and magnesium which are essential to fish for bone and scale formation. In present study, the maximum value of hardness was observed during summer (July) while the lowest value of hardness was observed in spring (March). According to Santosh and Singh (2007) the suitable range of hardness for fish is 30 mg/l to 180 mg/l. Species richness and fish abundance are significantly correlated with hardness. Similarly correlation between different environmental variables such as velocity and hardness, pH and hardness are significantly correlated.

Edds (1993), Kauamelan *et al.* (2003) and Pokharel *et al.* (2018) found fish abundance and species richness were high in downstream than upstream site in Riverine water bodies. In the present study the species richness was high in downstream sites, which could be attributed to the diverse physiography, turbidity, water velocity, temperature and various physicochemical characteristics and biotic interrelationship of fishes. Edds *et al.* (2002) and Pokharel *et al.* (2018) reported lower fish abundance and species richness in winter seasons and higher in summer and spring seasons in different Rivers. In the present study fish abundance and species richness were similar to the above mentioned observations, which could be attributed to the physiochemical characteristics, biotic community and hydrological regime.

5.3. Relationship between fish assemblage and environmental variables

Different environmental variables influences on fish health as well as diversity and distribution of fishes in water bodies. Among different environmental variables the temperature and DO are mostly responsible to the observed changes in species diversity and these variables are changes in fresh water assemblages according to seasons and elevations gradients. In the present study the amount of Do was highest in the summer and fish abundance was also highest in this season. The temperature as well as fish abundance were recorded less in winter season (Santosh and singh, 2007). Similar types of result was observed from running water of champman and Hall, London (Allen, 1995), from lakes of France and north-east USA (Irz *et. al*. 2007), from stream of the central Andes of Columbia (Jaramillo-Villa *et. al*. 2010). The most important environmental variables structuring the fish assemblages in the Pathariya river were water velocity, DO, pH, CO_2 and turbidity and fish assemblages structures mostly correlated with hardness, DO and temperature. Pokhrel *et al.*(2018) observed that the most important environmental variables were conductivity, water depth, free carbondioxide, ph, DO in Seti Gandaki river basin. The fish assemblages structures mainly correlated with free carbondioxide, water discharge, and stream size in North Tiaoxi River China (Koel and peterka, 2003). In consistent with our results, the fish assemblages structure of Kali Gandaki River were strongly correlated with the DO, pH, alkalinity, salinity and conductivity (Edds, 1993 and Duby, 2012).

6. CONCLUSION

The "spatiotemporal variation of fish assemblage structure in Pathariya River of Kailali district, Far western Nepal" were studied from September 2017 to August 2018 covering four different seasons. In the study period a total of 25 species were recorded from four different stations. The cyprinidae was common fish family which includes 59.21% while perciformes was a smallest fish family and covered only 9.58%. The *Puntius* and *Mystus* species were highly abundant while *Labeo* and *Ompok* species were less abundant. The highest Shannon winner diversity index (2.96) and highest species richness (22) were recorded in the station D. While lowest diversity index (1.67) and lowest species richness (6) was found in the station A. The fishes of the Pathariya River were biologically diverse and not uniformly distributed because the species were dependent on temperature, water velocity, transparency, DO, CO_2, pH, hardness and other environmental variables. The fish diversity was highest in the autumn and lowest in the winter seasons. The variations in fish assemblage structure in Pathariya River are probably related to habitat structure, altitude, season and several environmental variables. Variation in instream characteristics of the river are likely to be caused by natural variability of the ecosystems and harmful human activities such as deforestation, removal of woody debris, extraction of sand, cobble stones and construction of dam, agricultural activities and pollution.

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APPENDICES

APPENDIX 1. Correlation between fish and different environmental variables

	sp_rich	shan_win	temp	tran	velo	ph	do	co2	hard
sp_rich	1								
shan_win	0.98	1							
simp	0.91	0.98							
temp	0.21	0.16	1						
tran	-0.23	-0.2	-0.41	1					
velo	0.14	0.08	0.4	**-0.73**	1				
ph	-0.36	-0.32	-0.91	**0.5**	-0.46	1			
do	-0.05	-0.11	0.32	-0.47	**0.75**	-0.39	1		
co2	-0.17	-0.12	-0.97	0.41	-0.47	0.89	-0.42	1	
hard	**0.7**	**0.65**	**0.48**	-0.47	**0.49**	**-0.51**	0.17	-0.44	1

APPENDIX 2. Summarized table of data analysis

	sp_rich	shan_win	temp	tran	velo	ph	do	co2	hard
Minimum	6	1.673	15	7	0.03	7.5	6.56	6.32	150
1St Quartile	6.75	1.828	22	17.25	0.50	7.6	7.032	6.71	155
Median	8	1.948	26.5	40	0.66	7.75	7.67	7.285	158
Mean	10.06	2.13	24.38	40.25	0.85	7.81	8.029	7.933	158.3
Third Quatile	13.5	2.519	28	62	0.99	7.92	8.525	8.248	160.2
Maximum	22	2.966	29	77	2.16	8.4	10.66	11.85	170
Standard dev	4.7	0.41	4.71	26.52	0.64	0.26	1.32	1.75	5.08
Standard error	1.18	0.1	1.17	6.63	0.16	0.06	0.33	0.43	1.27

Photo Plate of collected fish species

Photo plate 1: *Puntius sophore*

Photo plate 2: *Puntius terio*

Photo plate 3: *Puntius ticto*

Photo plate 4: *Macrognathus armatus*

Photo plate 5: *Labeo calbasu*

Photo plate 6: *Labeo caeruleus*

Photo plate 7: *Cirrhinus mrigala*

Photo plate 8: *Cirrhinus reba*

Photo plate 9: *Barilius barila*

Photo plate 10: *Aspidoparia morar*

Photo plate 11: *Esomus Danricus*

Photo plate 12: *Cytnopharyngodon idellus*

Photo plate 13: *Acanthocobotis botia*

Photo plate 14: *Lepidocephalus guntea*

Photo plate 15: *Lepidocephalus menoni*

Photo plate 16: *Ompok bimaculatus*

Photo plate 17: *Mystus tengra*

Photo plate 18: *Mystus bleekeri*

Photo plate 19: *Mystus vittatus*

Photo plate 20: *Aorichthys seenghala*

Photo plate 21: Clarius batrachus

Photo plate 22: *Channa punctatus*

Photo plate 23: *Nandus nandus*

Photo plate 24: *Macrognathus aral*

Photo Plate of field and lab work