SPATIO-TEMPORAL VARIATION OF FISH ASSEMBLAGE STRUCTURE IN DOBHAN KHOLA OF PALPA DISTRICT, NEPAL

Submitted by

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Central Department of Zoology

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October, 2020

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 specifically acknowledged by reference to the author(s) or institution(s).

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RECOMMENDATIONS

This is to recommend that the thesis entitled **"SPATIO-TEMPORAL VARIATION OF FISH ASSAMBLAGE STRUCTURE IN DOBHAN KHOLA OF PALPA DISTRICT, NEPAL"** has been carried out by **Mina Ale** for the partial fulfillment of Master's Degree of Science in Zoology with special paper **Fish Biology and Aquaculture**. This is her original work and has been carried out under my supervision. To the best of my knowledge, this thesis work has not been submitted for any other degree in any institutions.

Date: 12 Feb 2020

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LETTER OF APPROVAL

On the recommendation of supervisor **"Prof Dr. Kumar Sapkota**, Central Department of Zoology, Tribhuvan University" this thesis submitted by Mina Ale entitled **"SPATIO-TEMPORAL VARIATION OF FISH ASSAMBLAGE STRUCTURE IN DOBHAN KHOLA OF PALPA DISTRICT, NEPAL"** is approved for the examination and submitted to the Tribhuvan University in partial fulfillment of the requirements for Master's Degree of Science in Zoology with special paper Fish Biology and Aquaculture.

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CERTIFICATE OF ACCEPTANCE

This thesis work submitted by **Mina Ale** entitled **"SPATIO-TEMPORAL VARIATION OF FISH ASSAMBLAGE STRUCTURE IN DOBHAN KHOLA OF PALPA DISTRICT, 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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LIST OF ABBREVIATIONS

Abbreviated form	Details of Abbreviations
APHA	American Public Health Association
CO_2	Free carbon-dioxide
DO	Dissolved Oxygen
FAO	Food and Agriculture Organization
DCA	Detrended Correspondence Analysis
RDA	Redundancy Correspondence Analysis
M	metre
Ml	Millilitre
pH	Hydrogen Ion Concentration
Mg/l	Milligram per litre
S.N.	Serial number
NaOH	Sodium hydroxide
Wtemp	Water temperature
Autmn	Autumn
Wintr	Winter
Sprng	Spring

ABSTRACT

The present study deals with the spatio-temporal variation of the fish assemblage structure in Dobhan Khola, Palpa district. The fish samples were collected from different sampling stations by using cast net during autumn, winter and spring season. A total of 14 species of fishes belonging to 3 orders, 5 families and 10 genera were recorded from Bhutaha, Beure, Jhyangla and Dobhan. Among the collected fishes Cyprinidae (50%) was recorded to be the most dominant family and followed by Cobitidae (29%), Balitoridae (7%), Sisoridae (7%) and Channidae (7%) respectively. *Garra rupecula* was the most common fish species followed by *Barilius bendelensis, Garra gotyla gotyla and Puntius terio.* The highest Shannon-Weiner diversity index value was recorded at station D (2.43) and lowest at station A (2.19). The maximum Species richness was found at station D (3.04) while minimum at station A (2.28). The highest Shannon-Weiner diversity (2.49) and Species richness (2.87) were recorded during spring season (May). The lowest Shannon-Weiner diversity (2.39) and Species richness (2.53) were recorded during winter season (October).The environmental variables such as water temperature, dissolved oxygen, free carbon-dioxide and pH were correlated with the fish assemblage structure. Redundancy analysis (RDA) revealed a significant relationship between fish assemblage, seasons and environmental variables.

1. INTRODUCTION

1.1. General Background

Fish assemblages are important biological indicators for the evaluation of the aquatic ecosystem (Gua *et al*. 2018).Basically, fish fauna of the river system of high land Nepal is palearctic, midland strictly Indo-Chinese, and low land Nepal is that of Indian (Sharma 2017).The water surface area of Nepal covers 0.1 percent of the total world water systems and fish diversity accounts 0.21 percent of total global fish diversity (Shrestha 1995).

Fishes are an integral component of streams which determine the distribution and abundance of other organisms in the ecosystem and are good indicators of the water quality and health of the ecosystem (Bijukumar 2000, McCormick *et al*. 2000).The diversity assemblies are affected by various components working at various temporal and spatial scales (Pease *et al*.2012, Presley *e.t al*.2010).Stream ecologists have been addressed relationship of assemblage schemes with abiotic (Mouillot *et. al*. 2007), biotic (Macarthur and Levins 1967), space (Peres-Neto and Legendre 2010) and stochastic impacts (Chase *et al*. 2011).Diversity assemblages are influenced by these factors autonomously, consecutively, or at the same time(Carvalho and Tejerina-Garro 2015).

The diversity of fish, the structure and species assemblages in waterways, springs and rivers are interdependent on many abiotic and biotic factors and these factors strongly determine the achievement or disappointment of fish collections in the waterways or streams or springs inside the scope of spatial scales (Minn 1989, Negi and Mamgain 2013).Freshwater habitats in rivers and streams are heterogeneous because of variations in altitudes, flow rates, dissolved oxygen, and physical substrate which provide food and shelter (Armantrout 1990).As a result, these types of freshwater habitats present a more diverse fauna with fish acting as prime indicators of the state of the ecosystem (Karr *et. al*. 1986). The structure and heterogeneity of the fish community are adequately exposed in tropical areas, compared to temperate, waterways, rivers and information on tropical fish communities is scarce (Nair *et al*. 1989, Arunachalam *et. al*.1997, Arunachalam and Sankaranarayanan 1999, Martin *et. al*. 2000). Riverine fauna show a high level of endemism, with most endemic fish species living in streams of river (Groombridge 1992, Kottelat and Whitten 1997).

The structure of the stream and river fish community is impacted by biological elements(Peterson and Rabeni 2001).For the regulation of fauna diversity and population the ecological components like temperature, salinity, and alkalinity assumes a vital role (Abdullah 2017).Marshall and Elliot (1998) noted significant correlation between various individual fish species and water temperature, salinity and dissolved oxygen. So that these parameters content of natural waters are essential for the existence of fish in the riverine system (Durmishi *et. al*. 2008).

The fish assemblage has been related to various hydrological factors such as rainfall (Kehat and Wyndham 1972). Poff and Allen (1995) used hydrological systems as a format for clarifying variation among fish assemblage in lotic ecosystems. A high flow is one of the significant regular procedures maintaining a network structure and system (Cummins and Spengler 1978, Resh *et. al*. 1988).Many studies have reported that an expansion in current flow leads to an expansion in the fish density of fish and diversity offish assemblage (Pegg and Pierce 2002, Aarts *et al*. 2004, Xenopoulos and Lodge 2006, Matono *et. al*.2012).On the other hand, low stream conditions represent negative effects on fish collection structure (Gehrke *et. al*. 1999, Pegg and Pierce 2002, Sagawa *et. al*. 2007).

Most commonly observed patterns in stream fish assemblage is the longitudinal zoning in the species diversity and composition, from headwater to downstream reaches (Horwitz 1982, Evans and Noble 1979, Schlosser 1982, Minckley 1984, Oberdorff *et. al*. 1993, Ostrand and Wilde 2002).Usually longitudinal gradients of rivers can be divided into upper, middle and lower reaches, and each area has its own characteristics of fish assemblage and structure (Schmutz *et. al*. 2000).Headwater stream regions are considered highly variable with environmental conditions and fish assemblage structure is controlled mainly by biotic factors and frequency of disturbances such as floods and drought (Echelle *et. al*. 1972, Matthews and Styron 1981, Tyler *et. al*. 1993).As a result, headwater fish assemblages are often deficient and can survive fish species that are capable of living in stressful conditions (Rahel and Hubert 1991).Then again, environmental conditions in downstream reaches less extreme, allowing the greater number of species to coexist (Ostrand and Wilde 2002).

Dispersion and abundance of fish assemblage is influenced by abiotic factors, for example, integrity of the riparian vegetation, the spatial differences in the diversity of the habitat and the structural complexity, the depth of the water, the velocity and substrate of the water and the spatial differences in the physical and chemical variables (Daga *et al*. 2012). Kollaus and Bonner (2012) concluded that current velocity, depth and vegetation are among the strongest factors explaining fish distribution. Biotic factors, such as predation or competition, show practically identical consequences for fish networks in both lakes and streams or river systems. However, these factors influence in lakes and streams are very unique in their relative significance in determining the composition of the fish community. These abiotic factors in lakes show variation in a vertical arrangement. In contrast, stream or river systems exhibit them in longitudinal arrangement (Jackson *et. al*. 2001).

Diversity and distribution of fish structure in rivers is at risk of many threats. Global freshwater biodiversity has higher rates of impacts than that of its seawater and terrestrial biodiversity (Dudgeon 2011, Liermann *et. al*. 2012, Yang *et. al*. 2016). Major threats in river systems are water pollution, over-exploitation augmented by various ecological changes, flow modification, destruction or degradation of natural habitats, rampant installation of industries, invasion of exotic fish species and global climate change that led to the risk of many species, specially endemic fish species (Dudgeon *et. al*. 2006, Rao *et. al*. 2014, Sharker *et. al*. 2015b, Siddek *et. al*. 2014).

1.2. Objectives of the study

1.2.1. General objective

- To explore the spatial and temporal variation of fish assemblage structure in Dobhan Khola of Palpa district, Nepal.

1.2.2. Specific objectives

- To determine the spatio-temporal variation in species composition and abundance.
- To describe the relationship between environmental variables and fish assemblage structure.

1.3. Significance of the study

Dobhan Khola is one of the important water streams of Palpa district which provides good habitat for fishes and other aquatic fauna and flora. There is no attempts have been made so far to explore the aquatic biodiversity in this khola. Nowadays the fishery resources are declining due to different environmental conditions, illegal fishing practices, habitat modifications and extraction of gravel and sand for developmental activities. So this study may serve to explore the diversity of different fish species of Dobhan Khola. And also encourage the local community to conserve the environment for healthy living.

2. LITERATURE REVIEW

2.1. Spatial variation of fish assemblage structure

Various biological and physiological factors operate the composition and diversity of fish assemblage in ecosystems. The change in species richness with altitude gradient is the most documented pattern for causing changes in species diversity in terms of spatial variation of fish structure (Rahbek 1995, Rosenzweig 1995, Lomolino 2001, Whittaker *et. al*. 2001, Sanders and Rahbek 2012). The fish diversity and distribution of river systems in temperate and tropical regions is correlated with altitude gradient (Bistoni and Hued 2002) and fish species richness is negatively related to the elevation. Hence, fish species richness is decreased with increased altitude (Jaramilo-Villa *et. al*.2010) or the altitude decreases with increasing stream size (Pouilly *et. al*.2006). In riverine system other variables such as current velocity, water depth, distance to source, substrate, stream width is also considered as important factors in fish distribution (Gormann and Karr 1978, Schlosser 1982, Moyle and Vondracek 1985, Bain *et al*.1988, Lobb and Orth 1991, Li *et. al*. 2012). The reason for decreasing fish species diversity at high altitude is the decreased land area (Lomolino 2001). Because of limited land area there is less amount of primary production of food resources and narrower niches of vegetation which directly or indirectly influence in the distribution of fish assemblage structure (Rahbeck 1995, Odland and Birks 1999, Grytnes 2003, Fu *et. al*.2006, Rowe 2009, Wang *et. al*. 2009, Sanders and Rahbeck2012, Askeyev 2015). In eastern extremity of Europe within the Tatarstan and Bashkortostan Republics of Russia, 42 species and two hybrid fish captured and Askeyev *et. al*. (2017) analyzed that species richness and Shannon index decreases as elevation gradient increases and found the highest numbers of fish at elevations of 250m and 500m whereas only five species at elevations above 650m.

Nepal is very rich in freshwater resources and these freshwater resources show glorious diversity of fish assemblage because of having an altitude from 60m to world highest peaks 8848m (Gurung, 2012). Geographically Nepal is divided into 3 regions: the Himalayan region at altitudinal gradient above 4800mfrom sea level covered with snow, the Sub-Himalayan or hilly region at elevation 500m to 4800m with cold water and the Terai region at altitudinal gradient 60m from sea level with warm water (Sharma 2017).The fish assemblage structure is differed along these elevations. In Terai, warm water fish such as *Labeo rohita*, *Catla catla*, *Cirrhinus mrigala* are indigenous carp with high commercial value (FAO 2012). The cold water consists of economically valuable indigenous fish such as *Neolissochelius hexagonolepis*, *Tor putitora*, *Tortor* (Gautam, 2015).

2.2. Temporal variation of fish assemblage structure

Temperature is the determinant factor for operating fish assemblage structure in riverine system (Buisson *et. al*. 2008). Water temperature directly influences on dissolved oxygen, salinity and alkalinity (Fisher and Willis 2000).Likewise, water temperature affects on the metabolism (Gillooly *et. al*.2001),breeding (Mills and Mann 1985), development and growth (Mann 1991, Wolter 2007) and behaviour (Taniguchi *et al*. 1998).At higher altitudes the water temperature shows thermal variation in streams or river systems causing lowered fish assemblage structure (Jacobsen 2008, Jaramillo-Villa *et al*. 2010).Temperature decreases with increasing altitudinal gradient (Koster 2005, Tockner *et. al*. 2009, Logez *et. al*. 2012). The diversity and distribution of fish species decrease with low water temperature and low precipitation at high altitude and increase with maximum temperature at lower land (Petts 2000). Therefore, fish assemblage structure differs due to water temperature variation. The environmental and temporal factors on fish distribution are of importance as it determines the water quality of a stream or river system. The fish distribution and assemblages is correlated with physicochemical variables such as dissolved oxygen, pH, free CO_2, conductivity including water temperature (Negi and Mamgain 2013). The physic-chemical variable i.e. dissolved oxygen is high at high land streams and low at low streams whereas the free CO_2, phosphorus, nitrogen concentration are higher at low land streams and lower at high altitude streams (Alexandre *et. al*. 2010). The fish assemblage structure of river streams correlated with different environmental variables. Pokhrel *et. al*. (2018) demonstrated that fish assemblage structure is strongly positively correlated with conductivity, free CO_2, phosphates, nitrogen and silicon compounds and negatively correlated with dissolved oxygen and pH.

Season has an impact on the distribution of fish composition in streams as environmental/ physico-chemical variables differed between seasons (Beugly and Pyron 2010).Seasonal variability is strongly correlated with streams fish assemblies (Jackson *et. al*. 2001). During the winter seasons, the water temperature decreases while the dissolved oxygen level is higher and the water temperature is higher, while the dissolved oxygen is lower during the summer seasons. Therefore, fish composition is more or less associated with seasonal variation (Li and Gelwick 2005, Trujillo-Jimenez *et al*.2010). Fish is ectothermic animal, increasing and decreasing temperature affects its growth (Donelson *et. al*. 2010). According to Bhatnagar *et. al*. (2004) the level of temperature for tropical fish is 28-32°C and less than 12°C is lethal for tropical fish while it is suitable for cold water fish species and more than 35°C is lethal to maximum number of fish species. Santosh and Singh (2007) and EL-Shafei (2016) suggested that the suitable water temperature for carp culture is between 24-30°C. The major fish species in lowland warm water consists of *Aristichthys nobilis, Hypophthalmichthys molitrix, ctenopharyn godonidella,* Cyprinus carpio, *labeo rohita* which can tolerate high level of temperature (Sharma 2008). The carps such as *Tor tor*, *Tor putitora*, *Neolissochilus hexagonolepis* , *Labeo angra* etc occurs in the hilly region while the *Schizothorax* spp. are dominated fish species in highland cold water streams (Edds 1993).

Although, number of studies related to the diversity of fish in different parts of Nepal, there is no study has been done on the fish assemblage structure in Dobhan Khola. Therefore, this study is aimed to investigate the spatio-temporal variation in fish assemblage structure of Dobhan Khola, Palpa district.

3. MATERIALS AND METHODS

3.1. Study Period

The field study was conducted from October 2018 to May 2019.Each sampling site was visited on the month of October, January and May.

3.2. Study Area

The present study was carried out in the Dobhan Khola that originates from the upper highland of Kachal of Palpa district Tinau Gaupalika about 24 km north-west from Butwal city. Dobhan Khola drains into the south and meets the Tinau River. The study area starts from Bhutaha at 27°46'02"N 83°24‘15"E to Dobhan at 27°44'40"N 83°27'41"E.

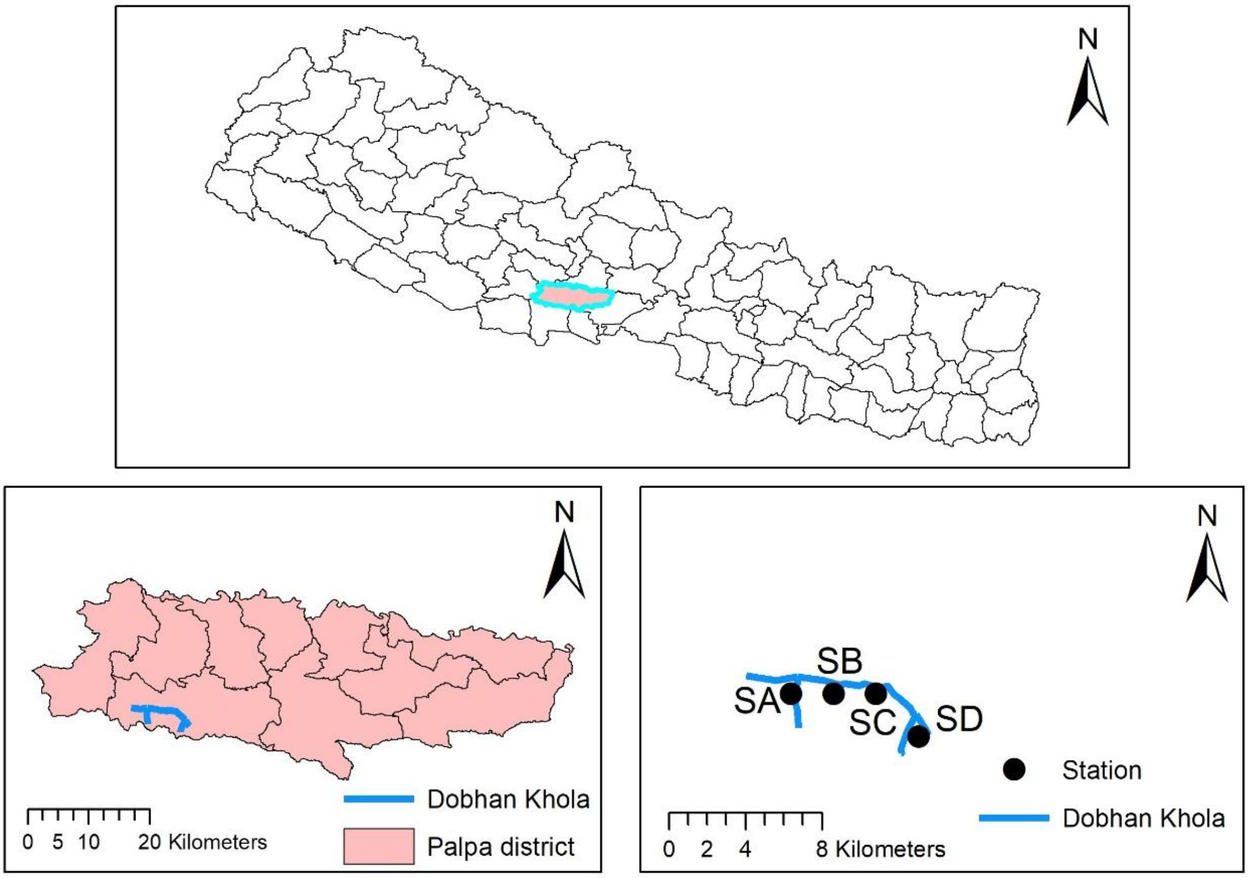

Figure1: Showing the study area

3.3. Selection of sampling stations

Before beginning the study work, a preliminary survey was done for fixing the study sites. The sites were selected to cover the faunal distribution of Dobhan Khola. The four sampling stations were selected based on the altitudinal variation, human settlement, confluence point etc. The selected four stations are as follows:

Station A-Bhutaha

Bhutaha lies in Tinau Gaupalika of Palpa district. It lies on 27°46'02"N 83°24'15"E and it has an elevation of 387m from the sea level. At this station boulders and pebbles are found and it has forest sites. The water colour of this station was crystal clear. The characteristic of the Dobhan Khola at this station was rapid.

Station B-Beure

Station B was selected at Beure about 2km down from Bhutaha. It lies on 27°45'50"N 83°25'20"E.It has an elevation of 341m from sea level. The river bed consists of sand, gravel, and bed-rocks. The water colour remains transparent. There was little human settlement and agricultural lands near this station.

Station C- Jhyangla

The sampling station C is situated about 3km south of station B and it lies on 27°45'45"N 83°26'44"E.It has an elevation of 300m.The river bed is mostly formed by pebbles, sand and mud.

Station D-Dobhan

Dobhan lies on 27°44'40"N 83°27'41"E and it is about 3km south from station C at an elevation of 283m from sea level. The water was bluish in colour. The river bed consists of silt, gravel and boulders. The Dobhan Khola is mixed with Tinau River from this station.

3.4. Analysis of environmental variables

Water samples of Dobhan Khola were collected during morning time (9:00 am to 10:00 am) and analyzed once every three months during field visits. The physico- chemical parameters were analyzed (APHA 1976, Adoni, 1985, Trivedy and Goel, 1986).

3.4.1. Physical analysis of water

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

3.4.1.1. Water Temperature

The temperature of water was measured by a standard mercury thermometer. The bulb was of thermometer was dipped inside the surface of water and reading was taken.

3.4.2. Chemical analysis of water

3.4.2.1. Hydrogen ion concentration

Digital PH meter was used in all the stations of Dobhan Khola to record the hydrogen ion concentration of water.

3.4.2.2. Free Carbon dioxide

To determine the free CO_2, 50ml of sample water was taken and few drops of phenolphthalein indicator were added. The obtained colorless solution indicated the availability of carbon-dioxide. Now this solution was titrated against standard alkali titrant (Sodium hydroxide 0.05N) to the slight pink end point. Free carbon-dioxide in the water sample was calculated using formula

$$\text{Free } CO_2 = \frac{\text{ml} \times \text{Normality of NaOH} \times 1000 \times 44}{V}$$

Where, V = Volume of water sample taken

3.4.2.3. Dissolved Oxygen (DO)

DO (mg/l) was analyzed by the Winkler titra-metric method. The sample of water from every sampling site was collected in a 300ml BOD bottle without bubbling. Then, 2ml of $MnSO_4$ and 2ml of KI was poured gently from the side of the bottle, then this mixture was shaken well so as to complete the reaction and the sample was left half an hour for the settlement of the precipitates (ppts.). About 2ml of conc. H_2SO_4 was added in the solution to dissolve the brown ppt. settled on the bottom.0.025N sodium thiosulphate was taken in the burette rinsed by the solution for titration. About 50ml of the mixture was taken on the conical flask and one or two drops of starch solution was added as an indicator. Then, the titration was done against the sodium thiosulphate solution, till the solution became colorless.

$$Dissovedoxygen = \frac{ml \times NofNa_2S_2O_3usedduringtitration \times 8 \times 1000}{V_2\,(\frac{V_1 - V}{V_1})}$$

Where, V= volume of $MnSO_4$ and KI

V1= volume of BOD bottle

V2= volume of part of titrant

3.5. Collection and Identification of fishes:

For the present study, the fishes were collected from each sampling station by employing the local fisherman and those fishes were collected by using cast net. Some fishes were caught by using hands. Cast net (15mm mesh size) was used for the collection of fishes.

The fish sampling was done at 8 am to 11am in every four sampling stations. The cast net was thrown 7 to 8 times for each station at about 400meter from each station. Before preservation, collected fishes were photographed with Nikon Digital Camera. After photography, the collected fishes were preserved in 10% formaldehyde. The specimens were taken to the laboratory of the Central Department of Zoology (CDZ) for identification. The identification was carried out with the help of taxonomic references Talwar andJhingran (1991), Shrestha (1981), Jayaram (2010) and Shrestha (2008).

3.6. Statistical Analysis of Ichthyofauna

3.6.1. Diversity Status

3.6.1.1. 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:

$$H' = -\Sigma(Pi) \times \ln(Pi)$$

Where, pi= n/N

Ni= No. of all individual species

N=Total no. of all individuals in the sample

ln= Logarithm of base e

3.6.1.2. Species richness index (d)

The species richness is calculated by using Margalef species richness (Margalef's1968)

Margalef richness index is designated by d, and calculated mathematically as, Margalef species richness (d) = S-l/lnN

Where,

S= Number of species

N = Number of individuals

3.6.1.3. Evenness index

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

$$E = H' / \ln S$$

Where,

H' = Shannon-Wiener's diversity index

S = Total no. of species in the sample

3.6.2. Data analysis

The relationship between environmental variables and species diversity were analyzed by redundancy analysis (RDA), a direct multivariate ordination method (ter Braak 1988a; ter Braak and Prentice 1988) based on a linear response of species to environmental gradients (Gauch 1982; ter Braak 1986; Palmer 1996) was applied by using vegan library in ‘R’ (Oksanen *et. al*. 2015).

4. RESULTS

4.1. Spatial and temporal variation of the environmental variables

4.1.1. Physical parameters of water

4.1.1.1. Water Temperature

The water temperature was noted to be lowest at station A(9°C) and the highest at the station D (25°C) (Figure 2).The average water temperature for the station A, station B, station C and station D were 15.50°C, 17.33°C, 17.17°C and 18.67°C respectively.

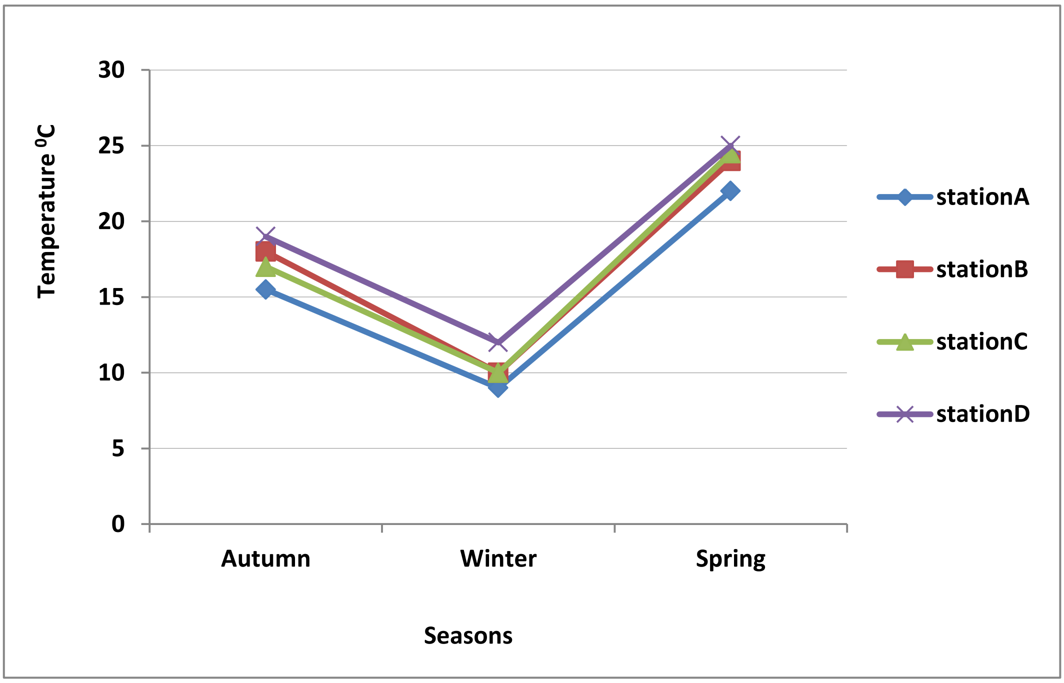

Figure 2: Variation of water temperature at four stations

4.1.2. Chemical parameters of water

4.1.2.1. Hydrogen Ion concentration (pH)

The pH of water of Dobhan Khola was noted slightly alkaline in all stations. The lowest value was noted 7 at station A during the Autumn season and highest value was 8.5 at station A, station C and station D during Winter season and Spring season respectively (Figure 3).

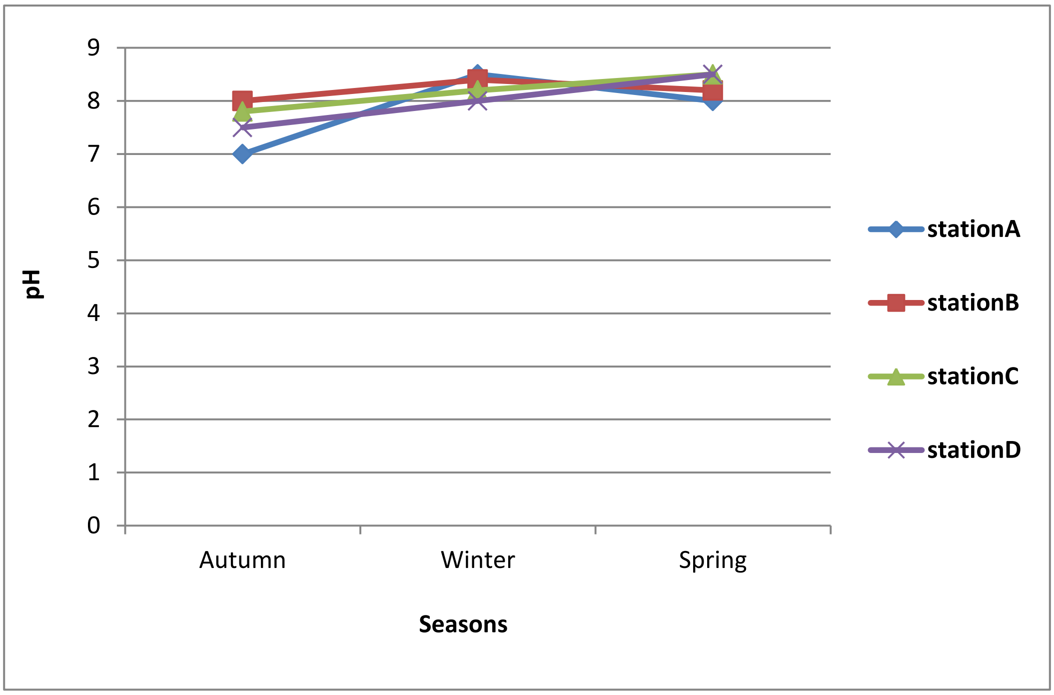

Figure 3: Variation of pH at four stations

4.1.2.2. Dissolved Oxygen (DO)

The maximum dissolved oxygen in water was recorded 10.5 mg/l at stationB during Winter season and the minimum was 7mg/l in Spring season at station D (Figure 4).

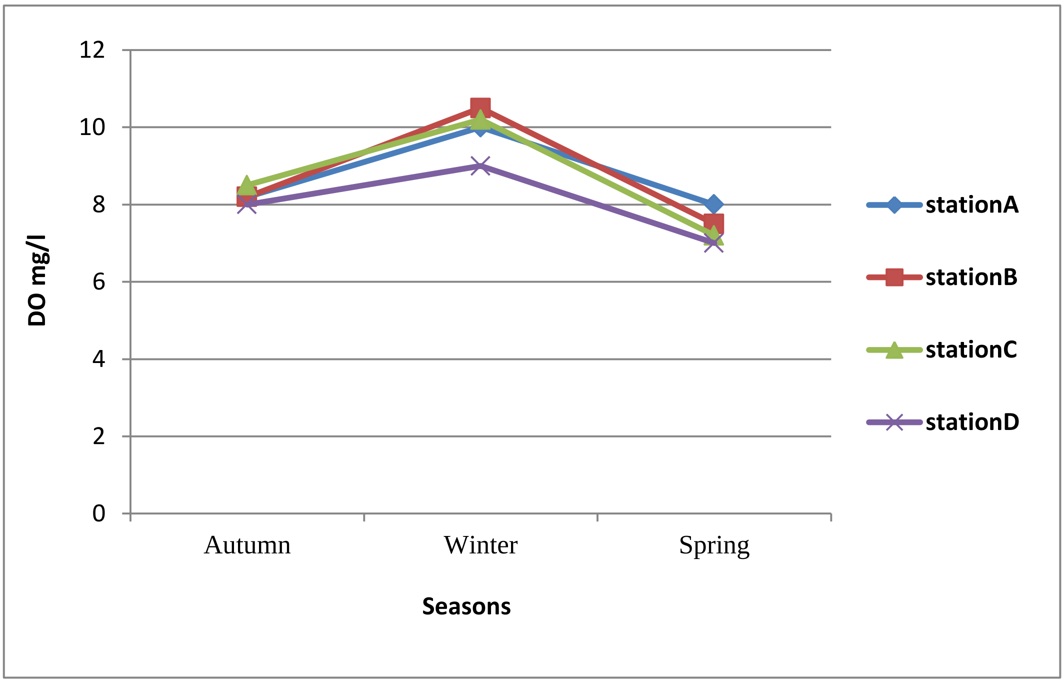

Figure 4: Variation of dissolved oxygen (DO) at four stations

4.1.2.3. Free Carbon-dioxide (CO_2)

The highest value of free carbon-dioxide was recorded 6.8mg/l in the Spring season at station D whereas the lowest value was 4mg/l at station A and station C in the Winter season (Figure 5).

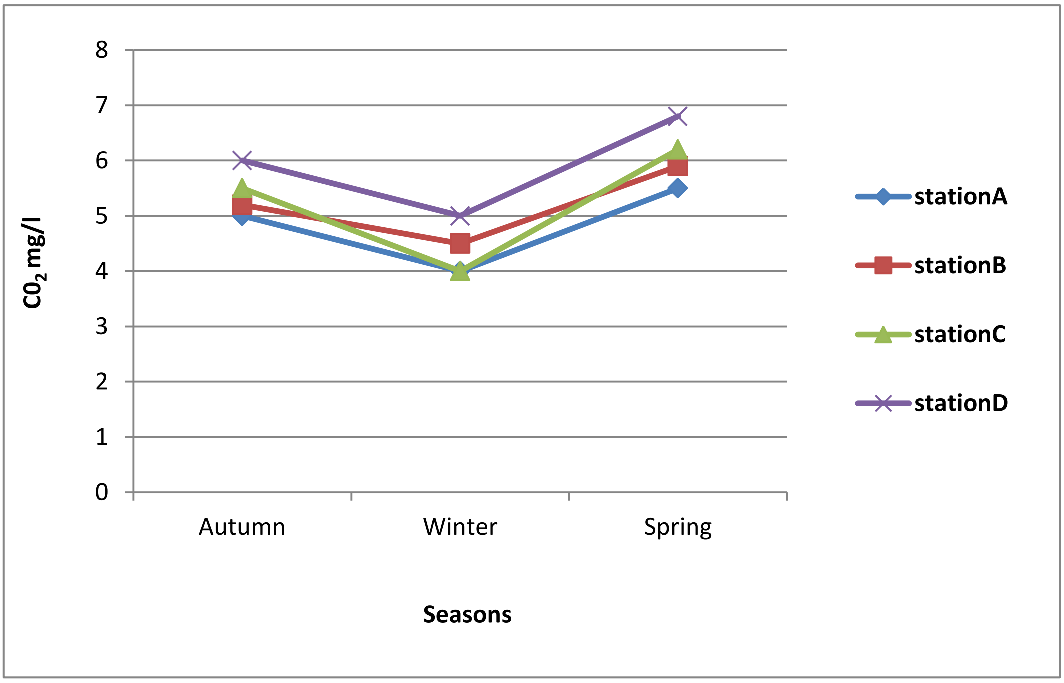

Figure 5: Variation of free carbon-dioxide (CO_2) at four stations

4.2. Spatial and temporal Variation of fish assemblage structure

4.2.1. Systematic Position of the Fishes

Fourteen different fish species were recorded during the present study from Dobhan Khola. The recorded fishes belonged to three orders, five families and ten genera. The collected specimens were identified and given proper systemic position as described after Jhingran and Talwar (1991), Shrestha (1981), Jayaram (2010) and Shrestha (2008).

Table1: Systematic position of fishes

S.N.	Order	Family	Scientific Name	Local Name
1	Cypriniformes	Cyprinidae	*Barilius barila*	Tiluwa
			Barilius bendelensis	Faketa
			Barilius barna	Faketa
			Neolissochilus hexagonolepis	Katle
			Garra gotyla gotyla	Buduna
			Garra rupecula	Buduna
			Puntius terio	Sidre

		Balitoridae	*Balitora brucei*	Chhepare machha
		Cobitidae	*Acanthocobotis botia*	Baghi
			Schistura horai	Goyti
			Schistura rupecula	Jikana
			Botia geto	Baghi
2.	Siluriformes	Sisoridae	*Glyptothorax pectinopterus*	Kapre
3.	Perciformes	Channidae	*Channa orientalis*	Hile

4.2.2. Distributional pattern and frequency occurrence of fishes in Dobhan Khola

From this study the highest dominant species of Dobhan Khola was *Garra rupecula* and the lowest frequency was of *Acanthocobotis botia*.

Table2: Distribution and frequency occurrence of fishes in Dobhan Khola

S.N	Name of fish	Autumn				Winter				Spring				Total	Frequency
		A	B	C	D	A	B	C	D	A	B	C	D		
1	*Garra gotyla gotyla*	2	0	3	3	2	1	3	3	1	2	3	4	27	10.8
2.	*Garra rupecula*	2	2	3	3	1	2	3	4	2	3	3	5	33	13.2
3.	*Barilius barila*	3	2	1	2	2	2	0	2	3	1	2	2	22	8.8
4	*Barilius bendelensis*	1	3	2	2	1	2	3	3	2	2	4	3	28	11.2
5.	*Barilius barna*	3	2	2	2	2	3	1	1	2	3	2	2	25	10
6.	*Neolissochilus hexagonolepis*	4	4	1	0	4	2	1	1	3	2	3	0	25	10
7.	*Glyptothorax pectinopterus*	1	1	3	1	1	1	3	0	0	2	1	2	16	6.4
8.	*Acanthocobotis botia*	0	0	0	1	0	0	0	0	0	0	0	1	2	0.8
9.	*Channa orientalis*	0	0	0	0	0	0	0	0	0	2	1	1	4	1.6
10.	*Puntius terio*	0	3	3	3	1	2	3	3	2	1	3	3	27	10.8
11.	*Botia geto*	0	0	0	1	0	0	0	2	0	0	1	1	5	2
12.	*Schistura horai*	1	1	1	1	0	2	1	0	2	0	1	1	11	4.4
13.	*Schistura rupecula*	1	1	1	1	1	2	1	2	2	1	1	2	16	6.4
14.	*Balitora brucei*	0	0	2	1	0	0	1	2	0	1	1	1	9	3.6
	Total													250	100

4.2.3. Order wise distribution of fish in Dobhan Khola

The study showed that Order Cypriniformes comprises 86% whereas Order Siluriformes and Perciformes comprise 7% as shown in figure 6.

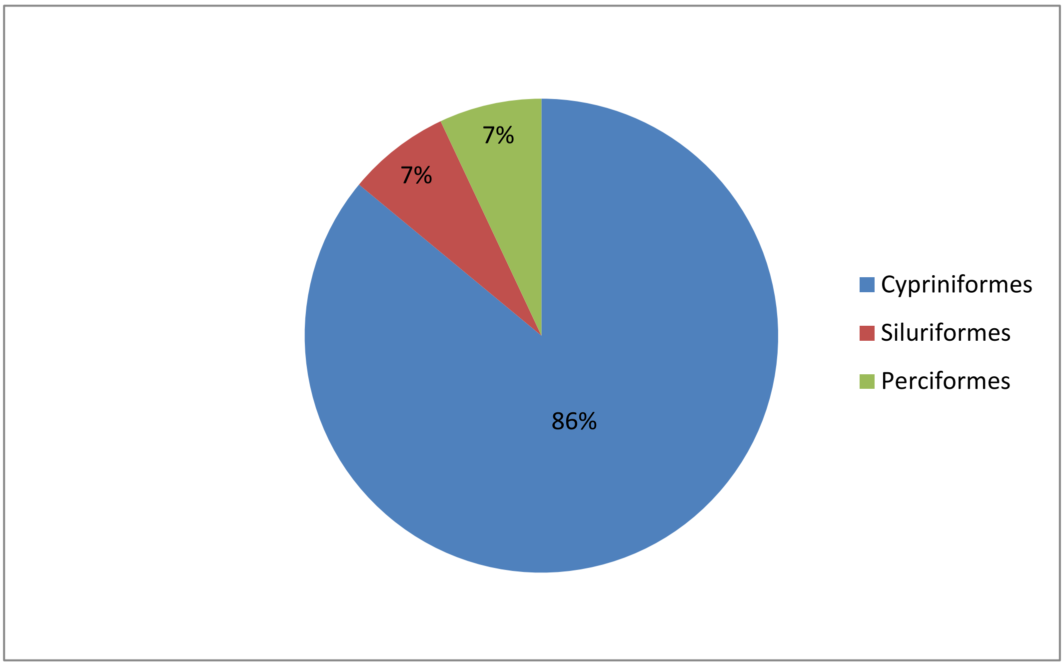

Figure 6: Orderwise fish species distribution in Dobhan Khola

4.2.4. Familywise distribution of fishes in Dobhan Khola

The result revealed that family Cyprinidae (50%) is the most abundant fish species followed by Cobitidae (29%), Balitoridae (7%), Sisoridae (7%) and Channidae (7%) which is shown in figure7.

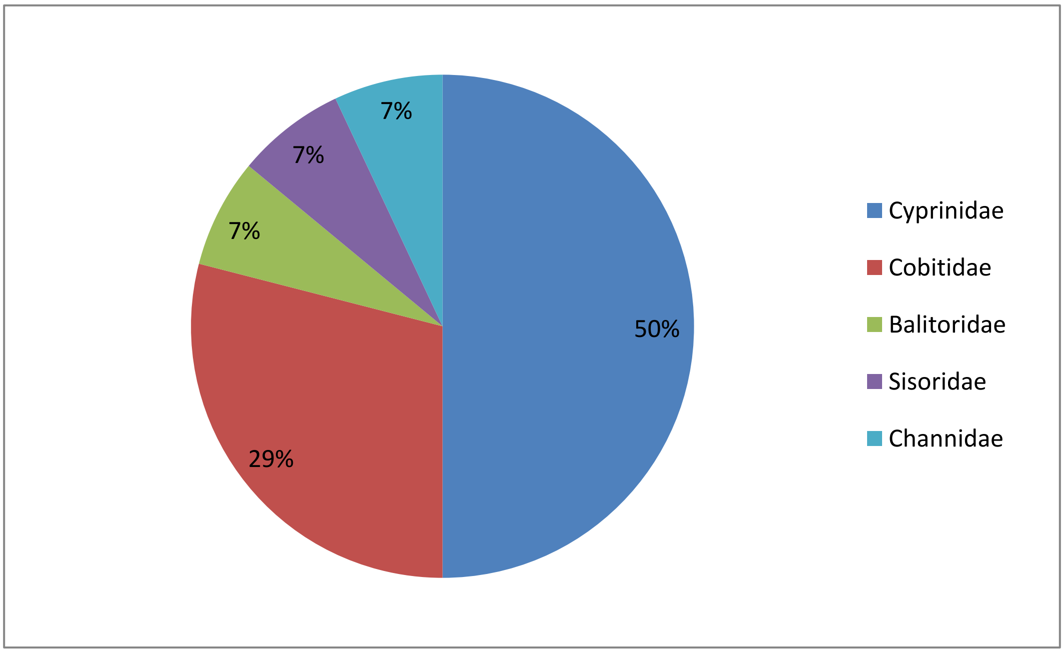

Figure 7: Family wise distribution of fish species in Dobhan Khola

4.2.5. Diversity status of fish of Dobhan Khola

The value of Shannon Weiner diversity index(H'), Evenness index(E) and Margalef species richness index(d) were calculated based on stations and months (Figure 8 and 9). Highest Shannon Weiner diversity index (2.43) was found in station D and the lowest (2.19) was found at station A. Similarly, the highest Shannon Weiner diversity index (2.49) was found in May (Spring) and the lowest (2.39) was found in January (Winter). No significant difference was found in the mean Shannon Weiner diversity index among the stations and months. Maximum Margalef species richness value 3.04 was found at station D whereas the minimum value 2.28 was found at station A. The highest Margalef species richness (2.87) was observed in May (Spring) and lowest (2.53) in January (Winter). Evenness index was observed highest (0.95) at station A and station B and lowest (0.92) at station D. Similarly, maximum evenness index (0.96) was found in January (Winter) whereas minimum (0.94) in May (Spring).

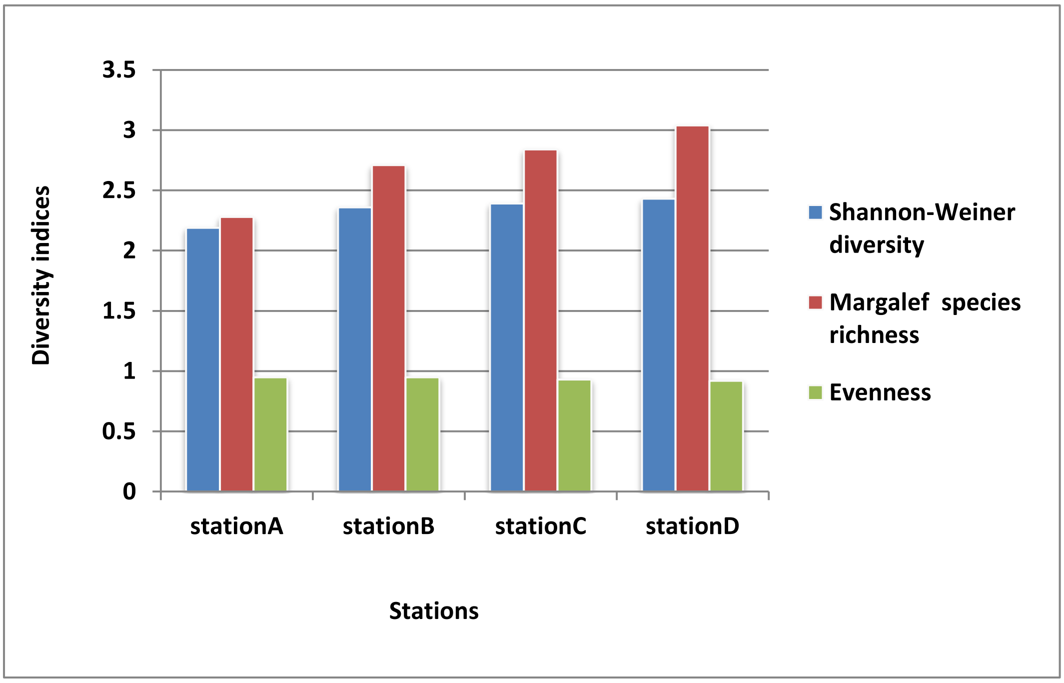

Figure 8: Spatial variation of fish diversity in Dobhan Khola

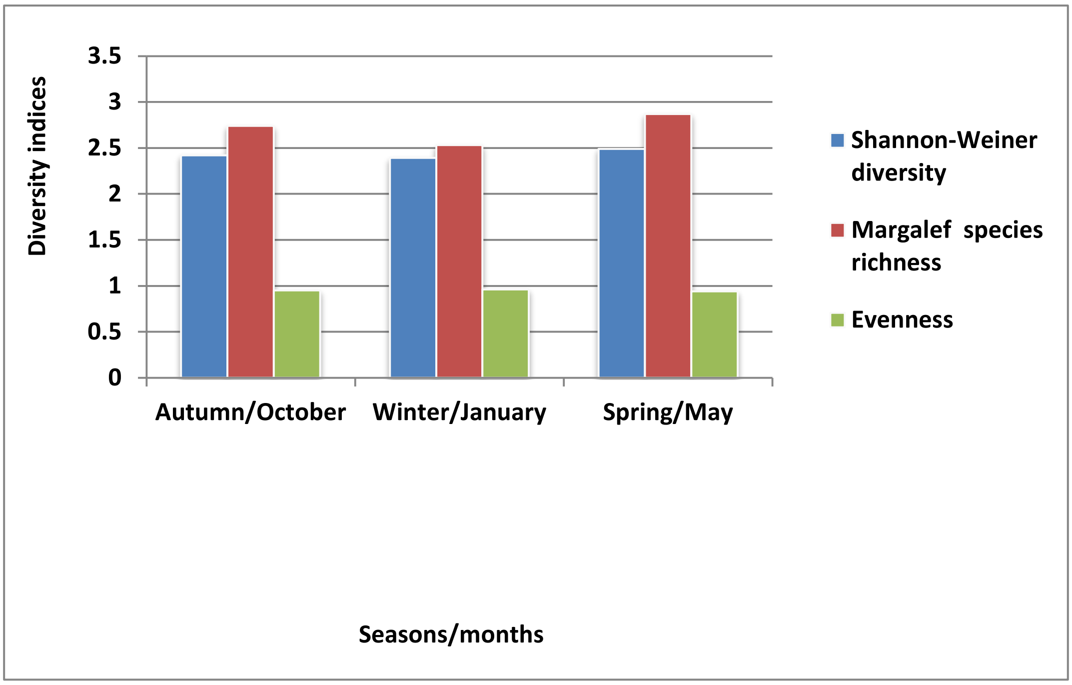

Figure 9: Temporal variation of fish diversity in Dobhan Khola

4.3. Relationships between stations, seasons, abundance fish species and environmental variables

4.3.1. Ordination

The axis length of the first axis of Detrended Correspondence Analysis (DCA) was obtained 1.05 followed by 0.93 in second DCA axis (Table 3). Thus Redundancy analysis (RDA) was done.

Table 3: DCA summary

	DCA1	DCA2	DCA3	DCA4
Eigen values	0.15	0.05	0.04	0.05
Decorana values	0.15	0.04	0.01	0
Axis length	1.05	0.93	0.65	0.82

The results obtained from redundancy analysis (RDA) were plotted in figure 10. The vector length of a given variable shows the importance of that variable in RDA analysis. In this analysis the longest vector length of free CO_2 at the second axis portrays a highly significant relation with *Garra rupecula* and *Garra gotyla gotyla* and station D. The vector length of dissolved oxygen at first axis showed significant relation with station B and *Neolissochilus hexagonolepis*. Likewise, the vector length of pH showed significant relation with station C and *Gylyptothorax pectinopterus*. The water temperature showed significant relation with station D and Spring season. Occurence of *Schistura horai* is associated with both Autumn and Winter seasons. Occurence of *Channa orientalis*, *Acanthocobotis botia*, *Schistura rupecula*, *Botia geto* are highly significant with spring season and station D. Occurence of *Barilius bendelensis* and *Barilius barna* are associated with station A. Station C is associated with *Glyptothorax pectinopterus*, *Puntius terio* and *Barilius bendelensis*. The ordination plot (fig: 10) divulged the negative relation between dissolved oxygen and free carbon-dioxide. The ordination plot revealed that the environmental variables of water temperature, dissolved oxygen, hydrogen ion and free carbon-dioxide are important variables for the present fish species.

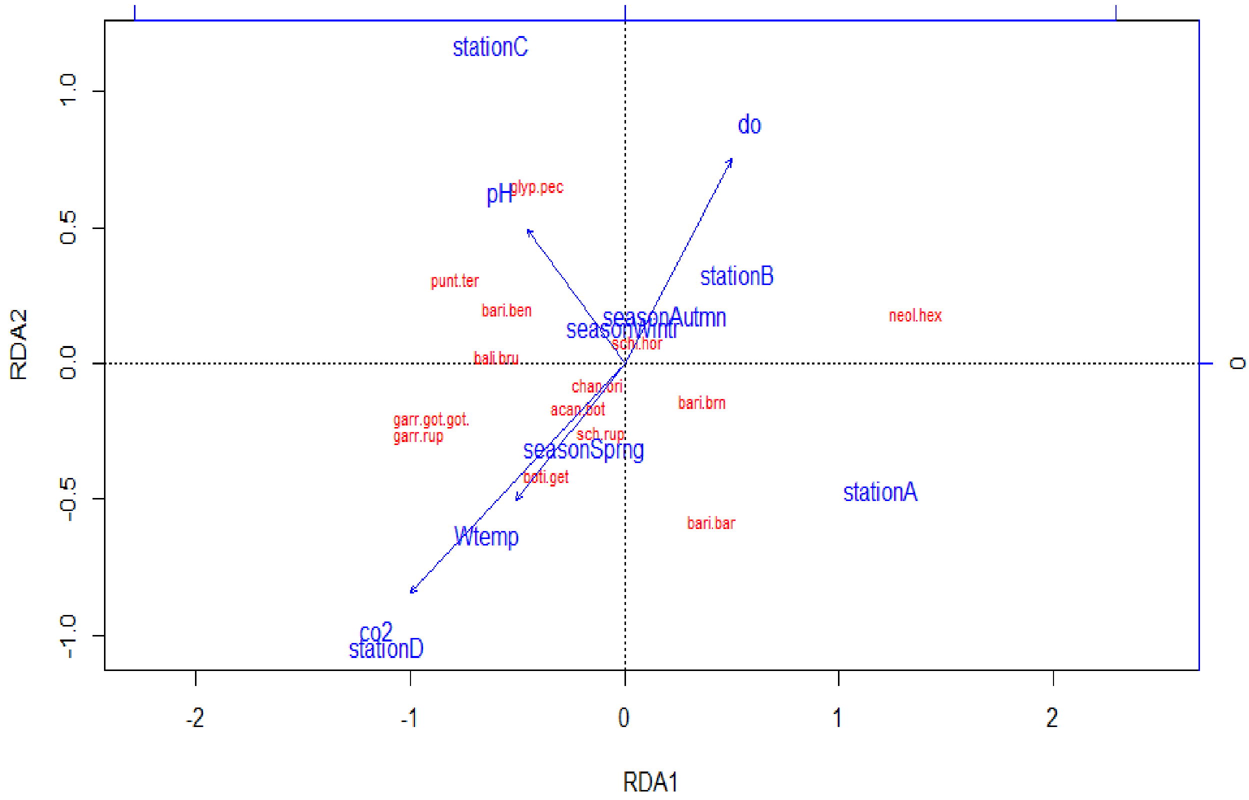

Figure 10: RDA analysis of species abundance, stations and environmental variables

5. DISCUSSION

5.1. Spatial and temporal variation of fish assemblage structure

In the present study a total of 14 species of fish were collected from four sampling stations of Dobhan Khola belonging to 3 orders, five families and ten genera. During the study period, altogether 250 fishes were collected. Among three orders Cypriniformes (86%) was the largest order followed by Siluriformes and Perciformes (7%). The fish species of the Cyprinidae family(50%)was dominated followed by Cobitidae (29%), Sisoridae (7%), Channidae (7%) and Balitoridae (7%). Among fish species, *Garra rupecula* was the most dominated species recorded from all stations throughout the study period. This dominated *Garra rupecula* with frequency 13.2% made contribution of the total species and was followed by *Barilius bendelens is* (11.2%), *Garra gotyla gotyla* (10.8%) and *Puntius terio* (10.8%). The least abundant fish species was *Acanthocobotis botia* recorded only at station D with occurrence of 0.8% and was followed by *Channa orientalis* (1.6%), *Botia geto* (2%) and *Balitora brucei* (3.6%).Sharma and Shrestha (2001) had found that Cyprinidae was the most dominant family in Tinau River. Similarly, Shrestha (2005) from Dano River, Shrestha et al. (2009) from Tamor River, Rijal (2015) from Tinau River, Mishra and Baniya (2016) from Melamchi River and Yadav (2017) from Singhiya River also reported that Cyprinidae as the most abundant family in their study areas. The fish assemblage structure more or less fluctuates with seasons. In the present study the fish abundance was low during winter and high during spring. Galacatos *et. al*. (2004) from Yasuni River, Li and Gelwick (2005),Gillette *et al*. (2006) from Midwestern U.S.A. River from floodplain River Texas, Jaramilo-Villa *et al*. (2010) from Central Andes of Colombia, Mohsin *et. al*. (2013) from Padma River, Hasan and Resen (2019) from Shatt Al- Arab River also reported high fish diversity in spring and low in winter season. This may be due to the fluctuation in different environmental variables such as low water temperature in winter and high in spring season, low temperature at high altitude while high temperature at low elevation.

The biodiversity index values (H') obtained from the present study are not so very high according to Shannon-Weiner diversity index values and do not exactly show the differences occurring among the stations either. The highest Shannon-Weiner diversity index was recorded in station D (2.43) and the lowest was recorded at stations A (2.19). The maximum species richness (3.04) was found in station D and minimum species richness was 2.28 in station A.

Species evenness is a measure of the relative abundance of the different species making the richness of a particular area. The maximum species evenness (0.95) was recorded at station A and station B while minimum was 0.92 recorded from station D.

5.2. Spatial and temporal variation of the environmental variables

Environmental or physicochemical variables such as water temperature, free carbon-dioxide, dissolved oxygen, pH are correlated with fish abundance and these variables change according to seasons and altitudes (Sharma *et. al*. 2007, Pokhrel 2011, Alexandre *et. al*. 2010, Negi and Mamgain 2013). In present study the highest water temperature was reported in spring at station D and lowest in winter season at station A. Mishra and Gupta (2015) also reported water temperature high during spring and low in winter. Water temperature was highest at station D where high fish abundance was found. Thus, water temperature is positively correlated with fish assemblage and affects the distribution of fish species. According to Bhatnagar and Singh (2010) suitable oxygen level is greater than 5 mg/l for fish. Fish can die if exposed to less than 0.3 mg/l of DO for a long period of time (Ekubo and Abowei 2011).Dissolved oxygen is an important factor which affects the distribution, diversity, physiology and behavior of fishes (Pokhrel *et. al.* 2018). Dissolved oxygen of present study reported highest (10.5 mg/l) in winter at station B and lowest(7 mg/l) in spring at station D. This showed that the range of dissolved oxygen in the river is good for most types of fish fauna. Most carbon-dioxide in the water is formed by the decomposition of organic matter and from respiration of the organism. Carbon-dioxide in surface water varies seasonally. According to Santosh and Singh (2007) free carbon-dioxide in water should be less than 5 mg/l for good production of fish. In present study the highest free CO_2 was 6.8 mg/l at station D in spring and lowest (4 mg/l) at station C in winter. Natural water of any water body may be neutral, acidic or alkaline which is an important environmental factor that influences the metabolic activities of fish. According to Bhatnagar and Devi (2013) ideal pH level is between 7.5 and 8.5 and above and below this is stressful to the fishes. The pH was found slightly alkaline from Dobhan Khola. Similar result was reported by Rijal (2015) from Tinau River.

In present study the fish diversity and species richness was found highest in lower streams at station D in comparison to higher streams at station A. This may be due to the availability of food resources, niches of vegetations, habitat structure of streams, different environmental/physicochemical variables and competition and predation. Similar results were found by Williams *et. al.* (1996), Lomolino (2001), Ostrandand Wilde (2002), Rowe (2009), Sanders and Rahbek (2012) and Askeyev *et. al*. (2017). Edds *et. al*. (2002), Nislow *et. al.* (2002) and Pokharel (2011) reported higher fish species richness and abundance in spring and summer and lower in winter season in various riverine systems. In the present study of Dobhan Khola fish abundance and species richness were found similar as mentioned above. This could be attributed to the seasonal variation which fluctuate the physico-chemical variables and biotic communities.

6. CONCLUSION

The spatiotemporal variation of fish assemblage structure of Dobhan Khola in Palpa district was observed from October 2018 to May 2019 for 8 months covering four stations. A total of 14 species were collected from four different stations of Dobhan Khola belonging to three orders, five families and ten genera. Dobhan Khola is acommon habitat for *Garra rupecula*, *Barilius bendelensis*,*Garra gotyla gotyla*, *Puntius terio*. Dominant order, family and genus were Cyprinifomes, Cyprinidae and *Garra rupecula* respectively. *Acanthocobotis botia* was least abundant recorded from present study. The highest fish diversity was recorded in spring and lowest in winter season. The results in the present study showed that spatial and seasonal variations in fish diversity were mainly related to environmental variables such as water temperature, dissolved oxygen, free carbon-dioxide and pH of the water. Variations in stream habitat are likely to be caused due to nature and harmful human activities such as deforestation, extraction of gravel, cobble, and sand for developmental activities, illegal fishing practices and pollution.

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APPENDICES

Appendix 1: Physico-chemical parameters in different four stations and seasons of Dobhan Khola

S.N.	Parameters	Autumn				Winter				Spring			
		station A	station B	station C	station D	station A	station B	station C	station D	station A	station B	station C	station D
1.	Water temp.(°C)	15.5	18	17	19	9	10	10	12	22	24	24.5	25
2.	DO(mg/l)	8.2	8.2	8.5	8	10	10.5	10.2	9	8	7.5	7.2	7
3.	Free CO_2 (mg/l)	5	5.2	5.5	6	4	4.5	4	5	5.5	5.9	6.2	6.8
4.	pH	7	8	7.8	7.5	8.5	8.4	8.2	8	8	8.2	8.5	8.5

Appendix-2: Photoplates of collected fish species

Photo plate 1: *Botia geto*

Photo plate 2: *Glyptothorax pectinopterus*

Photo plate 3: *Schistura rupecula*

Photo plate 4: *Acanthocobotis botia*

Photo plate 5: *Garra gotyla gotyla*

Photo plate 6: *Garra rupecula*

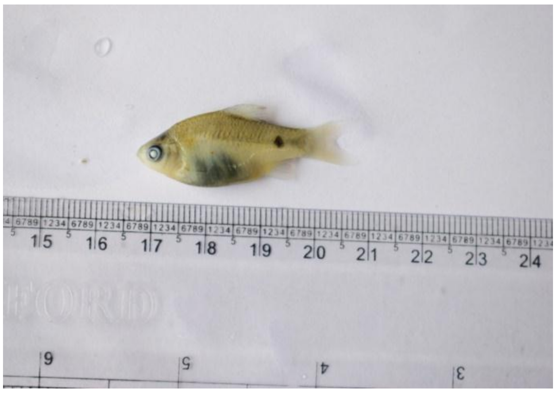

Photo plate 7: *Puntius terio* Photo plate 8: *Balitora brucei*

Photo plate 9: *Schistura horai*

Photo plate 10: *Channa orientalis*

Photo plate 11: *Barilius bendelisis*

Photo plate 12: *Barilius barna*

Photo plate 13: *Barilius barila*

Photo plate 14: *Neolissochilus hexagonolepis*