FISH DIVERSITY WITH RELATION TO WATER QUALITY OF DHUNGRE KHOLA, CHITAWAN

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

Submitted to:
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July, 2021

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 "Fish diversity with relation to water quality of Dhungre Khola, Chitwan" has been carried out by Sunil Gautam for the partial fulfilment of Master's Degree of Science in Zoology with special paper Fish Biology and Aquaculture. This is his 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.

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LETTER OF APPROVAL

On the recommendation of supervisor "Prof. Dr. Kumar Sapkota" this thesis is submitted by Mr. Sunil Gautam, entitled "Fish diversity with relation to water quality of Dhungre Khola, Chitwan" is approved for the examination in partial fulfilment 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 Mr. Sunil Gautam, entitled "Fish diversity with relation to water quality of Dhungre Khola, Chitwan" has been accepted as a partial fulfilment for the requirements of Master's Degree of Science in Zoology with special Fish Biology and Aquaculture

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Sincerely,

Sunil Gautam

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LIST OF ABBREVIATIONS

Abbreviated form	Details of Abbreviations
BOD	Biological oxygen demand
DCA	Detrented correspondence analysis
BOD	Biological oxygen demand
CCA	Conical correspondence analysis

ABSTRACT

Species diversity is acknowledged as foremost trait of ecosystem functioning. Seasonal variation of fish assemblage structure with physico-chemical parameters in Dhungre River were studied based on Stratified random survey conducted during September 2019 to May 2020. Three various seasons each with three different stations were selected for data collection. Shannon’s diversity index, the Simpson index and evenness index have been used to access the diversity. To explore the fish assemblage structure, fish samples were collected in seasonal basis from three sites of the River by using cast net and gill net. A total of 2200 individuals belonging to 7 orders, 11 families, 20 genera and 26 species were collected from Kathar, Botte and Bachauli of Dhungre River. The most abundant species were *Puntius sophore* followed by *Puntius ticto* and *Schistura devdevi*. The topmost Shannon’s diversity index (2.66) and Simpson index (0.81) were recorded during autumn and winter season respectively. For the station-wise diversity, station 1 had the greatest Shannon's diversity index (2.89) and Simpson index (0.85) of fish. The CCA analysis showed that the fish assemblage was highly correlated with temperature. On the other hand, velocity and DO negatively correlated with carbon dioxide and slightly correlated with pH. Present study could be accountable for further exploration of fish diversity, water parameters or other relevant topics in future. Imposing fisheries regulations to reduce human activities are inevitable to sustain multitudinous fisheries.

1. INTRODUCTION

1.1. Background

Riverine ecosystems have suffered from intensive human interference over the last century, resulting in habitat loss and degradation, and as a result, many fish species have become critically endangered, particularly in rivers where freshwater creatures are in increasing interest. Freshwater biodiversity is declining at an alarming rate, which is consistently above that estimated for terrestrial ecosystems (Dudgeon et al. 2006; Reid et al. 2019). Fishes are the group of vertebrates which share many common characters that helps them to adapt in the various aquatic ecosystem. There are at least 27,800 species globally, of which 10,000 are freshwater fishes (Shrestha 2008). The spatial and temporal fluctuation of river fish assemblage structure occurs at scales ranging from microhabitat to basin, and from diel to decadal or longer (Adams et al. 2004). According to the Florida Museum of Natural History, there are 27650 species of extant fishes, 41 % of which are freshwater and account for less than 0.01 % of Earth's volume. Over two-thirds of freshwater fish belongs to single clad Otophysi (i.e. minnows, characins, and catfish). In the Indian subcontinent, there are more than 2500 species with approximately 930 species belonging to the freshwater ecosystem (Jayram 2013). Fish form almost half of the total number of vertebrates in both fresh and marine aquatic ecosystems (Gupta 2006).

Although theory and studies firmly support the relevance of various stressors in determining biodiversity, their influence at biogeographically important spatial scales has hardly been studied (Gieswein et al. 2017; Ryo et al. 2018). Ideally, such studies should address local furthermore as regional or landscape level conditions using an equivalent analytical framework (e.g. types of habitats, water quality, and evaluation procedures) for a more accurate understanding of parameter effects. In this regard, the exploration of within station and between stations diversity changes in species composition has special relevance, since only their joint evaluation can reveal unequivocally how anthropogenic stressors influence spatial patterns in biodiversity (Heino 2011). However, studies that examine the effects of multiple stressors on various diversity components in a single study are few. An exception, for instance, is that of the study of (Göthe et al. 2015). Overall, freshwater organisms exhibit a wide range of responses to multiple stressors, which may be attributed to biogeographic/taxonomic differences in stressor effects and/or methodological differences in assessing stressor-response relationships (Olaya-Marn et al. 2012; Stendera et al. 2012;

Jackson et al. 2016; Lange et al. 2018). Recently, a new concept and metric, called zeta diversity in ecology have been introduced for characterizing spatial variation in species composition of multiple assemblages (Hui and McGeoch 2014; McGeoch et al. 2019). Freshwater fish diversity enables the calculation of how diversity is arranged across various spatial scales within the same framework. In brief, zeta diversity is that the mean number of species shared by i number of websites, with i mentioned because of the zeta order (McGeoch et al. 2019). Diversification of fish fauna has some crucial impacts, as within the organic phenomenon both directly and indirectly to stabilize the aquatic ecosystems (Polis and powerful 1996).

Many researchers investigated Nepal's accessible freshwater fish fauna, which has been evaluated by (Bhagat 1979a, 1984b; Menon 1974; Majupuria 1998; Shrestha 1991, 1994) and Shrestha 1995). These explorers presented location-wise checklists of the freshwater fish fauna of different regions of Nepal, with particular references to places, districts, zones, regions, rivers, streams, lakes, and so on. Different water quality parameters with the diversity, distribution, and abundance of species an unbreakable relationship have been established. Catfish and larvivorous fishes were found to be residing and schooling in the shallow and polluted water. Likewise, the type of water bottom, bed substrate, depth of water bodies, the density of water, and temperature along with its velocity played a vital role in the diversity and distribution patterns of the fish species (Dufendorf 2006). This study seeks to execute an influential plan to find a diversity of fish in the Dhungre River and determine the relationship between the assemblage of fish and water standards in this river system. Similarly, endeavors to explain the parallel relations among seasonal variations and fish diversity.

Nepal has more than 6000 rivers. When compared to the size of our nation, the quantity of rivers demonstrates that we have abundant water resources. Other than rivers there are streams, brooks, canals, reservoirs, ponds, and lakes. Despite being rich in water resources study on fish diversity, habitat, reproduction, biology, and ecology of fish are lacking. Why is that? As yet, researchers have conducted their work in limited rivers, streams, and lakes only. The geographical structure is one the most challenging obstacle of our country for any kind of headways. Other agendas like rustic, transportation problems and insufficient funding are pile on for the exploration.

1.2. Justification of the study

One of the most important studies for a country like ours is the study of variety in connection to habitat kinds and water quality. The seasonal fluctuation and types of habitat for fish, as well as their diversity, are well understood through this study. Furthermore, the fish species confirmation of Nepal has pointed out bigger problems because of lack of field observation, meticulous species identification, and irregular survey on fish diversity, ambiguous photographs and deposition of specimens. There is still a significant deal of uncertainty about the taxonomic characteristics of several Nepali fish species. Habit and habitat of many fish are still not known. On this account, the present study was set in motion to fulfil all these missing parts to some extent and I surmise that, this study will be convenient for commencers as well.

1.3. Objectives

a. General Objectives

- To investigate the fish diversity with relation to water quality in Dhungre Khola, Bhandara.

b. Specific Objectives

- To explore the fish diversity of the Dhungre Khola
- To assess the water quality parameters from Dhungre Khola
- To analyze the influence of water quality on fish diversity in Dhungre Khola

2. LITERATURE REVIEW

Riverine ecosystems have suffered from intensive human interference over the last century, resulting in habitat loss and degradation, and as a result, many fish species have become critically endangered, particularly in rivers where freshwater is in great demand. Especially in rivers where there is a high demand for freshwater. The basic reasons are habitat degradation and fragmentation (Cuizhang et al. 2003), water abstraction, manufacturing companies and personal utilization (Szollosi-Nagy 2004; Ricciardi and Rasmussen 1999; Gibbs 2000; Dawson et al. 2003), non-native species inclusion (Copp et al. 2005), contamination (Lima-Junior et al. 2006), and global climate change repercussions (Leveque et al. 2005; Mas-Marti et al. 2010). Freshwater fish, in fact, are one of the most endangered taxonomic groups (Darwall and Vie 2005) due to their great sensitivity to quantitative and qualitative changes in aquatic behaviours (Laffaille et al. 2005; Kang et al. 2009). As a result, they are frequently employed as a bio-indicator for assessing water quality, river network connection, or flow regime (Chovance et al. 2003). The dynamics of water flow bodies determined by the climate and topography (Allan 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. These structural alterations have a significant impact on diversity and fish assemblages based on altitudinal variation (Joccobsen 2008).

The northern Andes area has the greatest diversity of mountain fishes, with 37% of 220 species being endemic, and every 1000 m rise in altitude results in the loss of 19 species in Columbia (Rodriguez et al. 2016; Jaramillo et al. 2010). According to research, the richness of fish species decreases monotonically as the longitudinal gradient increases (Jaramillo et al. 2010; Juna et al. 2015). The assemblage of riverine fishes frequently changes over species composition from headwater to main stream (Schlosser 1982, Gelwick 1990, Edds, 1993). In Nepal, the diversity and species richness were also decreased with increased in elevation (Shrestha 2008). 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, zooplankton 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. The 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). Warm-water fish species such as *Labeo, Mystus, Puntius,* and *Channa* predominate in rivers of warm water, while *Labeo, Catla, Cirrhinus*, and Carps are significant fish species utilized for aquaculture in Nepal's Terai area since these species can live in high temperatures (Rai et al. 2008). While the fish species *Schizothorax, Gara, Glypothorax,* and *Pseudocheneis* predominate in cold water rivers in the hilly region, they have a limited capacity to withstand high temperatures (Petr et al. 2002). The cold water fish species such as *Neolissocheilus hexagonolepis, Schizothoraichthys spp, Schizothorax spp*, and *Tor spp* 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). The daily fluctuation of temperature is high at highland area in comparison to low land area (Bussion et al. 2008). The physico-chemical and microbiological characteristics of water described the quality of water. According to (Bhandari and Nayal 2008) the physico -chemical 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 communities and physico-chemical parameters including temperature in rivers and lakes are fluctuating according to seasons (Mehner et al. 2005).

3. MATERIALS AND METHODS

3.1 Study Area

The Dhungre River starts from north of Piple mostly from headwaters being mixed at different points. Hence, flows from north to south contributing to major East Rapti River. A river basin is characterized by a humid sub-tropic region and also characterized by the influence of the prevailing monsoon. The river section lies in Bhandara, Khurkure, Parsa, Khaireni, and Sauraha which is about 30 km long originating from Lothar in the Chitwan district The study area for this research covers 3 Km of river length starting from Kathar to Bachauli, Chitwan.

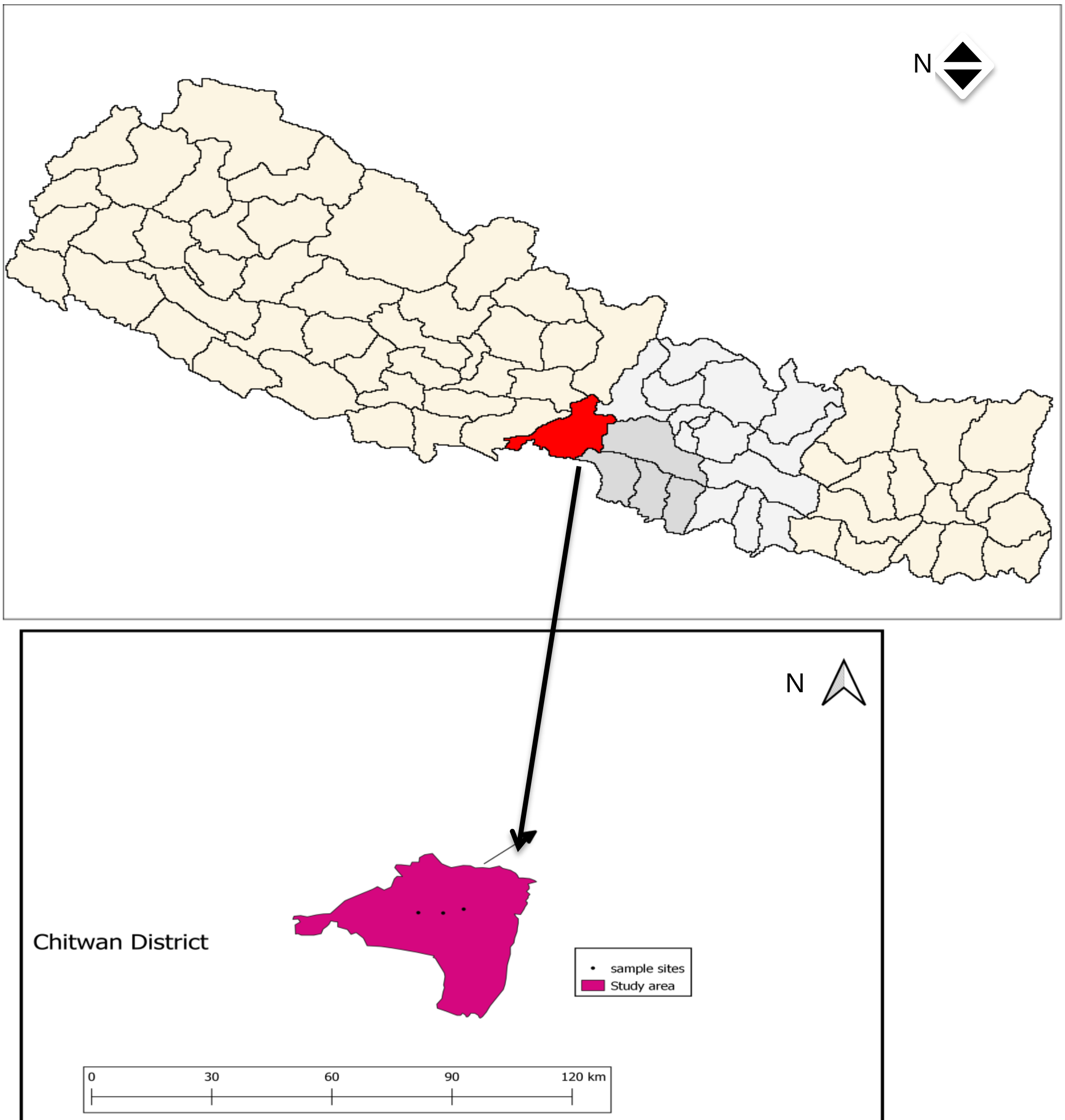

Figure 1 : Map of Nepal showing Sampling sites and Dhungre

3.2. SAMPLING SITES

There were three sampling sites in total. All the sites were at a distance of 1 km from each other. These sites were selected on the basis of physical division, human approach, and river confluence points. These are illustrated as:

a. Site 1- Kathar (290m length covered, 27°35'23.4"N 84°33'36.6"E). It is located at Khurkhure, Bhandara ward having moderate velocity of water.

b. Site 2- Botte (322m length covered, 27°34'58.5"N 84°32'29.1"E). Chitwan National Park has created a buffer zone in the center of Tarauli to prevent over-exploitation of vulnerable aquatic species. Khairani Municipality has a lower human approach and a shallower water velocity than other sites.

c. Site 3 - Bachauli (310 m length covered, 27°34'44.9"N 84°31'30.3"E). Being located at Bachhauli ward, Khairani Municipality with less human approach and shallow water velocity than other sites.

3.3.MATERIALS

Cast nets of monofilament type composed of hemp fibers with a mesh size of 0.5cm, 6m diameter, and 6-kilogram weight, as well as 1 cm, 6 m diameter, and 8 kg, were in plentiful uses. Mesh size of 1 cm was used in a higher volume (depth more than 4 feet) of water and smaller mesh size 0.5 cm used in a lower volume (depth below 4 feet) of water.

Besides, dragnet from local fishermen made by local mosquito net of mesh size of 2.3 cm was also used for collecting smaller fish. Furthermore, hand catching, trapping methods with the help of locally made traps of wood and fiber, hooking, and other local methods were used.

The tools like Nikon D5600 24.2MP DSLR camera was used for photography. Similarly lab thermometer, digital pH meter, BOD bottles, Petri disc, table tennis ball, manganese sulfate, sulphuric acid, sodium thiosulfate, and potassium iodide were used for analysis of different physical and chemical parameters.

3.3.1. Fish Sampling

The collections were carried out in three seasons, from autumn September 2019 to spring May 2020. Each site was visited 3 times in a season and thus a total visit to 3 sites was 9 times in a season. About 300 m of each site was surveyed. The fish were sampled during the morning time (7 a.m. to 12 p.m.). The fish were caught with the assistance of local fisherman using a cast net and dragnet with a mesh size of 2.3 cm and dimensions of 2 X 6 m, weighing 5.5 kg in total. Besides these methods other gears like scoop net having a diameter of 50 cm was used. For the trapping method, local traps were constructed of wood and fiber as if a fish swims inside through its opening, it cannot get out and those traps were placed at two locations at each site for 1 hour considering the turbulent flow of the river. Hooks were placed for 1 to 2 hours at each site according to river depths and human approach and handpicking collections were done at every site wherever feasible. The fishing gears were operated within 300m length at each sampling station from 7 a.m. to 12 a.m. Explaining in more detail, for each site a total of 25 to 30 throws (based on necessity) were made by cast net and 12 hauls for gill net to catch the fishes. For estimation of the abundance of fishes, two pass removal methods (Seiber and Le 1967) were used. Each removal pass at each side of the river within a pre-determined length of 300m included hunting first upstream then downstream for 20 minutes approximately.

3.3.3. Fish identification and preservation

The collected fishes were identified on the site and at the CDZ lab using (Shrestha 1981, 1994; Shrestha 2008; K.C. 2010 and Talwar et al. 1995). When feasible, the captured fish were photographed in their fresh state on the spot. They were properly labeled and stored in a 10% formalin solution in plastic jars for future investigation in the CDZ lab.

3.4. WATER QUALITY ANALYSIS

3.4.1. Physical parameters

Only, two physical parameters were observed which were temperature and velocity. For the measurement of temperature a lab thermometer was used. Temperature was measured by dipping the thermometer inside the water at 5 different strata in a sampling site and average value of temperature was noted.

Velocity was measured by floatation method i.e. a ping pong ball was allowed to float in the water surface at fixed distance and time taken by the ball to cover the distance was noted. The experiment was repeated for five times and average of time was calculated. Then the velocity of water was calculated by the formula:

$v = \frac{d}{t}$ Where, '**V**' denotes velocity, '**d**' denotes distance and '**t**' denotes time taken.

3.4.2 Chemical parameters

Different chemical parameters like Hydrogen ion concentration (pH), dissolved oxygen and free carbon dioxide was determined by using standard measuring kits and titration. The pH was measured by dipping the pH meter into the water after buffering its bulb. Similarly, 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 millilitres 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 thiosulfte solution (0.025N) and the calculation was carried out using formula:

$$\text{DO}\left(\frac{\text{mg}}{\text{l}}\right) = \frac{\text{ml}\times\text{normalilty of titrant}}{v_2\left\{\frac{V_1 - V}{v_1}\right\}} \times 800$$

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.

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

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

Where, V = volume of the water sample taken (ml)

3.5. STATISTICAL ANALYSIS

a) Shannon's diversity index was used to find out the fish diversity.

- It is calculated by using following formula:
- $H = -\sum_{i=l}^{k} P_i \, log \, P_i$

Where:

H = The Shannon diversity index

P_i = fraction of the entire population made up of species i

S = numbers of species encountered

∑ = sum from species 1 to species S

Note: The power to which the base e (e = 2.718281828.......) must be raised to obtain a number is called the **natural logarithm** (ln) of the number.

b) Simpson diversity index

Simpson diversity index was used to see dominance index. In the Simpson index, p is the proportion (n/N) of individuals of one particular species found (n) divided by the total number of individuals found (N), Σ is still the sum of the calculations, and s is the number of species.

Simpson diversity index $(\mathrm{D}) = \frac{1}{\sum_{\mathrm{i=l}}^{\mathrm{s}} \mathrm{p_i^2}}$

c) 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.

d) Multivariate analysis

The relation between fish diversity and environmental variables were analysed by detretended correspondence analysis (DCA) and canonical correspondence analysis 10 (CCA) method (Tar Break 1988a, 1988b and Prentice 1988) by using vegan library in ‘R’ (Oksanen et al. 2019).

4. Results

4.1 Fish assemblage structure

Table 1: Fish species and their order,family and genera

SN	ORDER	FAMILY	SUBFAMILY	GENUS	SPECIES	LOCAL NAME
1	Cypriniformes	Cyprinidae	Cyprininae	*Puntius*	*sophore*	Shidri
2					*ticto*	Shidri
3				*Labeo*	*dero*	Rahu
4					*boga*	Rahu
5			Danioninae	*Barilius*	*barila*	Thople
6					*vagra*	lamphe
7				*Opsarius*	*tileo*	mamphi
8				*Esomus*	*danricus*	Tito shidra
9		Cyprinidae		*Brachydanio*	*rareo*	
10		Cyprinidae		*Chela*	*laubuca*	
11		Cyprinidae	Barbinae	*Acrossochellus*	*hexagonolepis*	kattiale
12		Cobitidae	Nemacheilinae	*Schistura*	*devdevi*	Chiple gohera
13		Cobitidae		*Lepidocephalichthys*	*guntea*	Patte gohera
14	Tetraodontiformes	Tetraodontidae		Tetraodon	cutcutia	Fuwa
15	Beloniformes	Belonidae	Beloninae	*Xenentodon*	*cancila*	Chuche bam
16	Siluriformes	Bagridae		*Mystus*	*tengara*	Tyangra
17					*bleekeri*	Chatti
18					*vittatus*	Tyangra
19				*Olyra*	*longicaudata*	
20		Sisoridae	Sisorinae	*Glyplothorax*	*alkanandi*	
21		Siluridae		*Wallago*	*attu*	
22	Synbranchiformes	Mastacembelidae		*Macrognathus*	*pancalus*	Chiple bam
23	Cichliformes	Cichlidae		*Oreochromis*	*mossambicus*	Tilapia
24	Perciformes	Anabantidae	Anabantinae	*Anabas*	*testiduens*	chebti
25		Channidae	Channinae	*Channa*	*gaucha*	Hile
26					*puncatus*	Bhoti

Overall, 26 species of fish belonging to 7 orders, 11 families and 20 genera were collected from three various stations of the Dhungre river during three different seasons (Table.1). A total of 2200 fish individuals of 26 species were noted. *Puntius sophore* was most dominant fish followed by *Puntius ticto*, *Schistura devdevi*, and *Lepidocephalichthys guntea*. On the other hand, *Oreochromis mossambicus* was least abundant species followed by *Wallago attu* and *Tetraodon cutcutia* . All the fishes recorded in all the sampling stations and their frequency distributions are given in (Table. 2).

4.1.1 Species wise distribution of fish in Dhungre River.

There was higher abundance of *Puntius sophore* which was followed by *Puntius ticto*, *Schistura devdevi, and Lepidocephalichthys guntea*. Similarly, *Oreochromis mossambicus*, *Wallago attu*, *Tetraodon cutcutia* and *Labeo boga* were least abundant species (Figure 2).

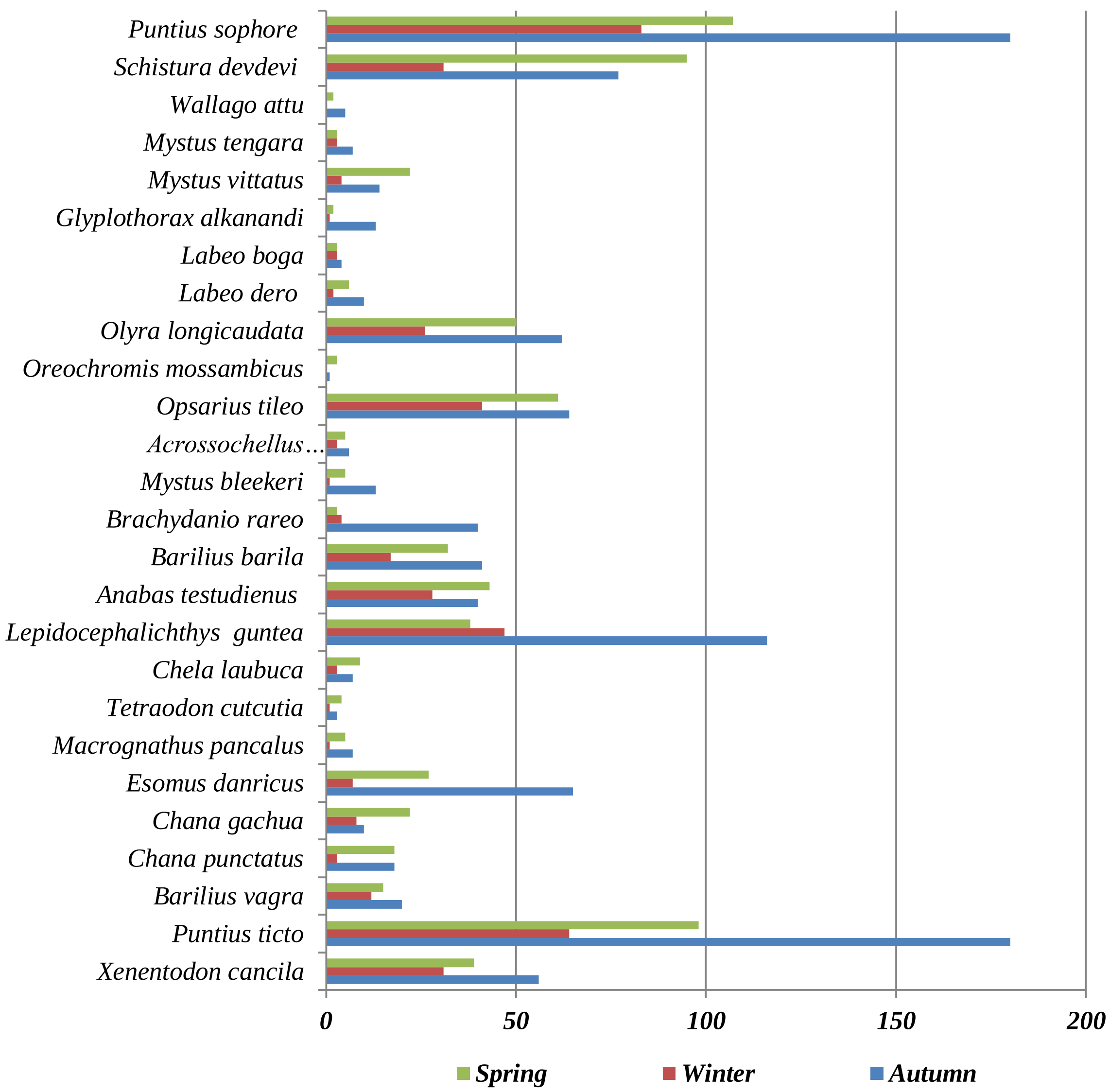

Figure 2: Distribution fish of Dhungre River.

4.1.2. Diversity Index and Evenness

The sampled data was analyzed using Shannon's diversity index, Simpson index, and evenness. The data was classified into seasonal and strata-wise (station-wise) patterns, and the resulting data was input into R statistical software to produce Shannon's index, Simpson's index, and evenness (Figure 3 and Figure 4, respectively).

4.1.3. Seasonal diversity and distribution

The season-wise diversity structure of fish was computed and evaluated by determining Shannon's diversity index, Simpson index, and evenness values (Figure 3). Shannon's index had the greatest value in the spring season, which was 2.70, followed by autumn 2.66 and winter 2.52. Similarly, the Simpson index was greater in spring with a value of 0.81, while the evenness was determined to be 0.42 in winter, 0.41 in spring, and 0.38 in autumn.

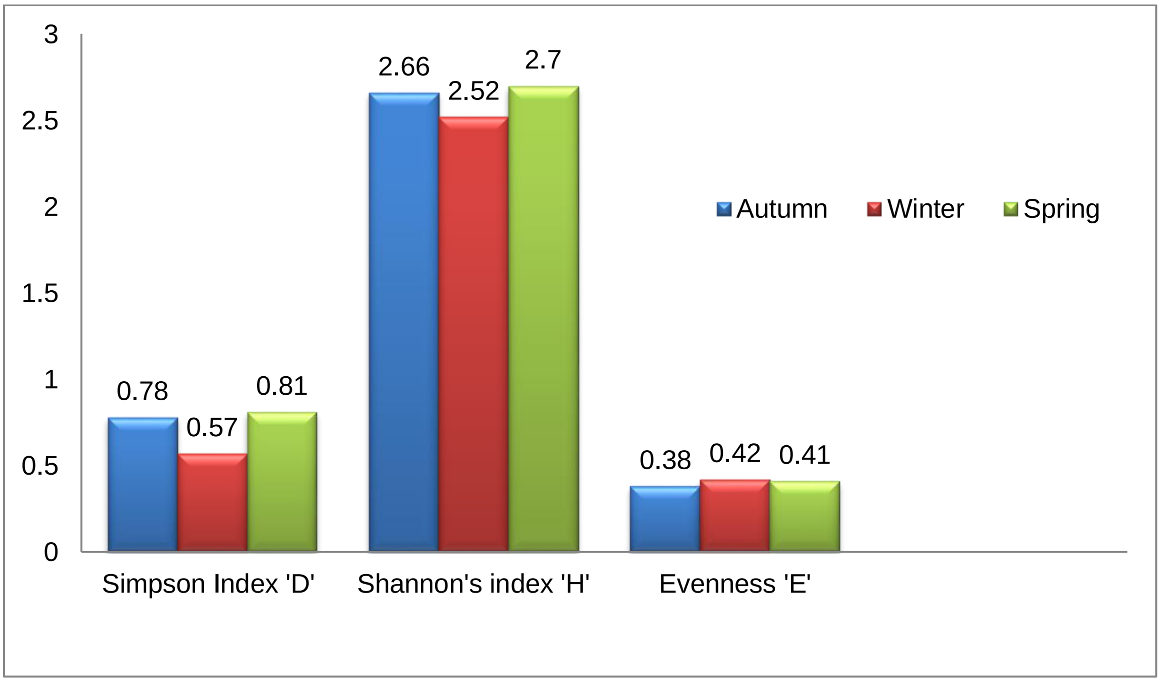

Figure 3: Season wise diversity and evenness pattern of fish species.

4.1.4. Station Wise diversity and Distribution

The station 1 showed high value of 'H' i.e. Shannon index valued to be 2.89 followed by station 2 and station 3. Similarly Simpson index and evenness was also high at station 1 having value of 0.85, 0.73 at station 2 and 0.71 at station 3. While in case of evenness Station 1 and station 2 had the identical values of 0.48 which descended a little to 0.44 at station 3.

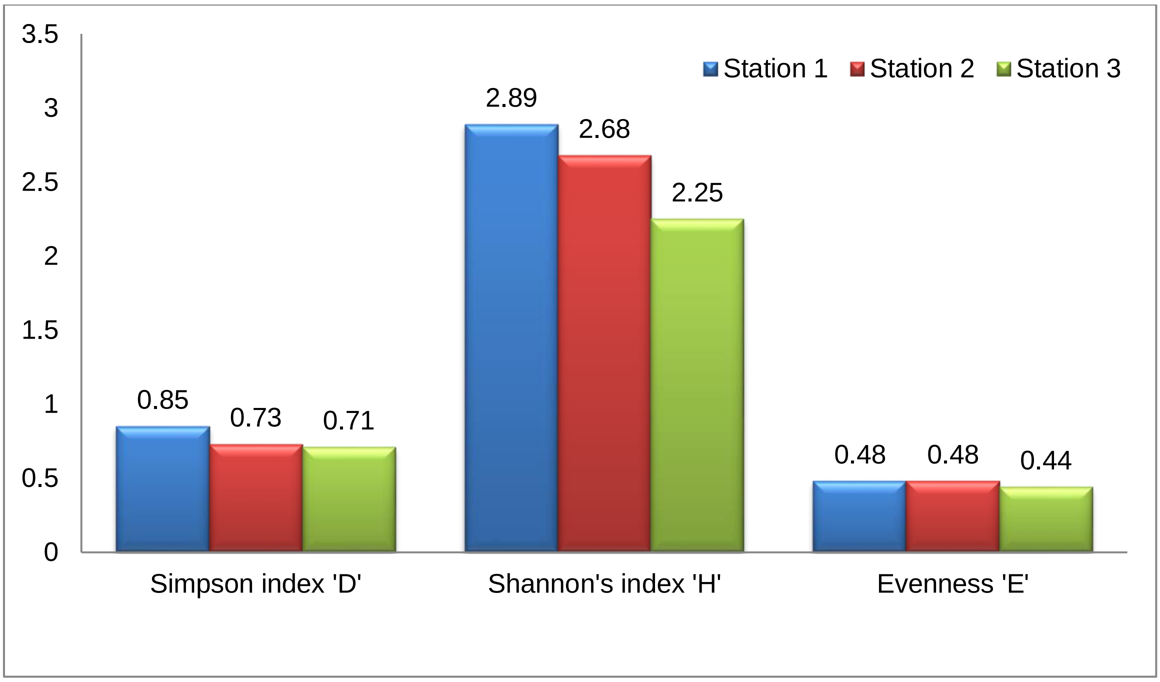

Figure 4: Station wise diversity and evenness pattern of fish species.

4.2. Seasonal and station wise variation of physical and chemical parameters

During the data collecting procedure, several physical and chemical parameters such as temperature, DO, free carbon dioxide, velocity, and pH were monitored. Among the different physical and chemical parameters temperature was seemed indirectly proportional to both dissolved O_2 and free CO_2. Which means the increase in temperature decreases the value of DO and CO_2 and vice-versa. In case of pH it remained quite stable throughout all three seasons. Meanwhile, velocity of water flow did not change noticeably from autumn season to winter season but ascended dramatically in spring season.

4.2.1. Temperature

The highest record of temperature was noted 21 ℃ at station 2 and station 3 during autumn season. Surprisingly, station 1 in spring season also had temperature similar to station 1 and station 2 of autumn that is 21 ℃. On the other hand, lowest temperatures 15 ℃ was recorded in winter season at station 1 and station 3. The average value of temperature recorded during the whole study period was 18.66 ℃ (Figure 5).

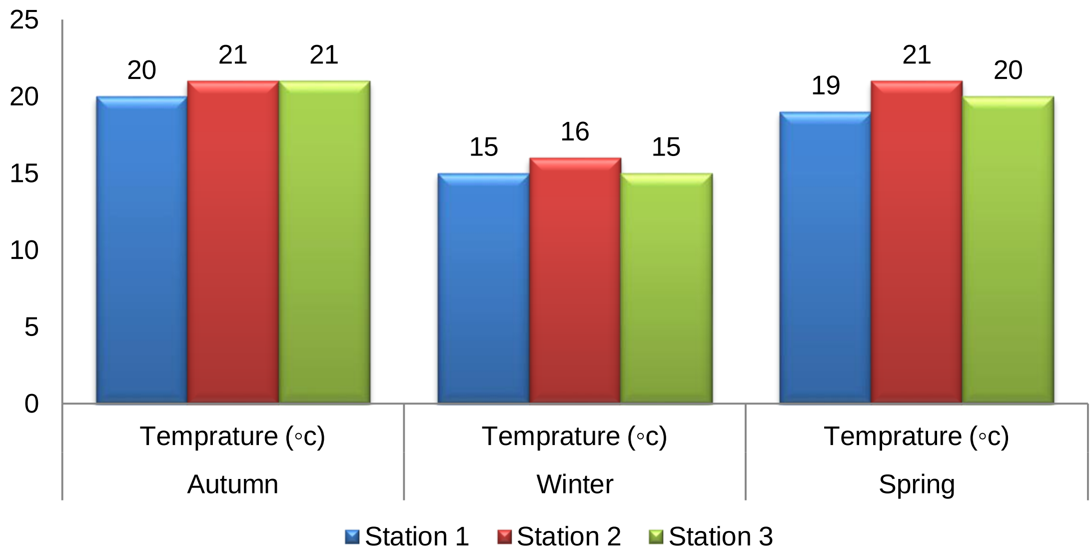

Figure 5: Seasonal variation of water temperature at different stations

4.2.2. Dissolve Oxygen (DO)

The level of dissolved oxygen was found to be highest 9.6 ppm at station 1 during spring season and lowest 5.82 ppm at station 2 during autumn season and average value of dissolved oxygen was found to be 7.53 ppm during the study period (Figure 6).

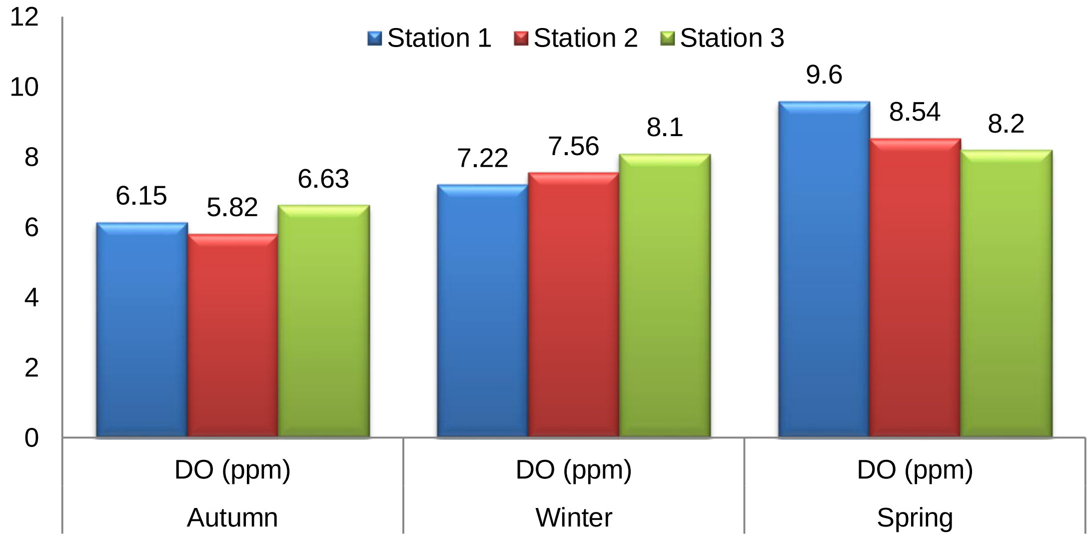

Figure 6: Variation of DO at different seasons

4.2.3. Velocity

Velocity was measured at stations 3 and 1 with the greatest value of 1.1 m/s during the winter and spring seasons, respectively, and the lowest value of 0.25 m/s during the autumn season. During the study period, the average velocity was 0.61 m/s (Figure 7).

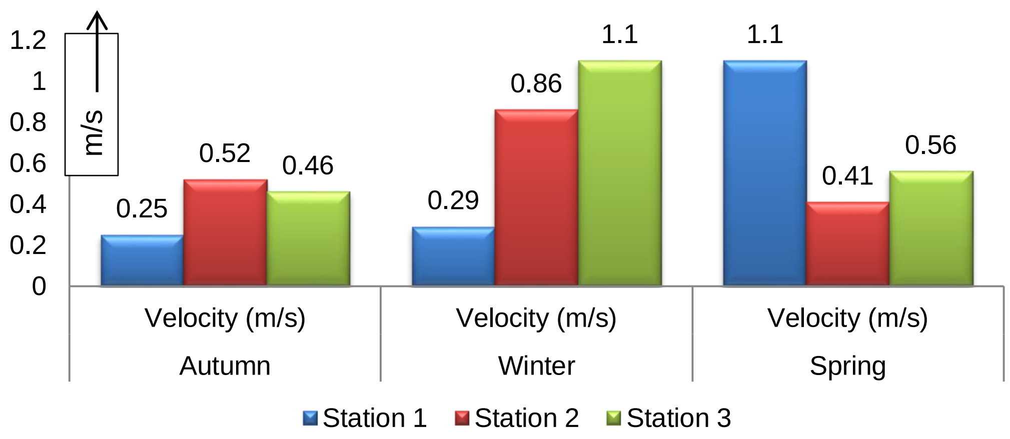

Figure 7: Variation of Velocity at different seasons

4.2.4. Hydrogen ion concentration pH

pH of the water was recorded highest 7.63 during autumn and spring seasons both at Station 2 but lowest pH was 7.12 at station 1 during winter season. The average value of pH during the study period was 7.31 (Figure 8).

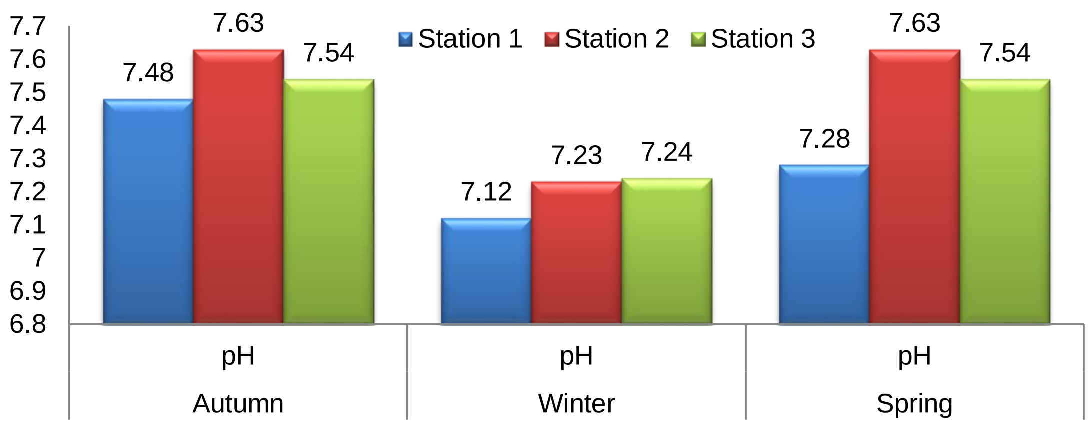

Figure 8: Variation of pH at different stations

4.2.5 Free carbon dioxide

The highest value of free carbon dioxide was recorded to be 11.73 ppm at station 3 during spring season and lowest value was recorded to be 8.22 ppm at station 1 during autumn season. The average value of free carbon dioxide was 9.32 ppm during the study period (Figure 9).

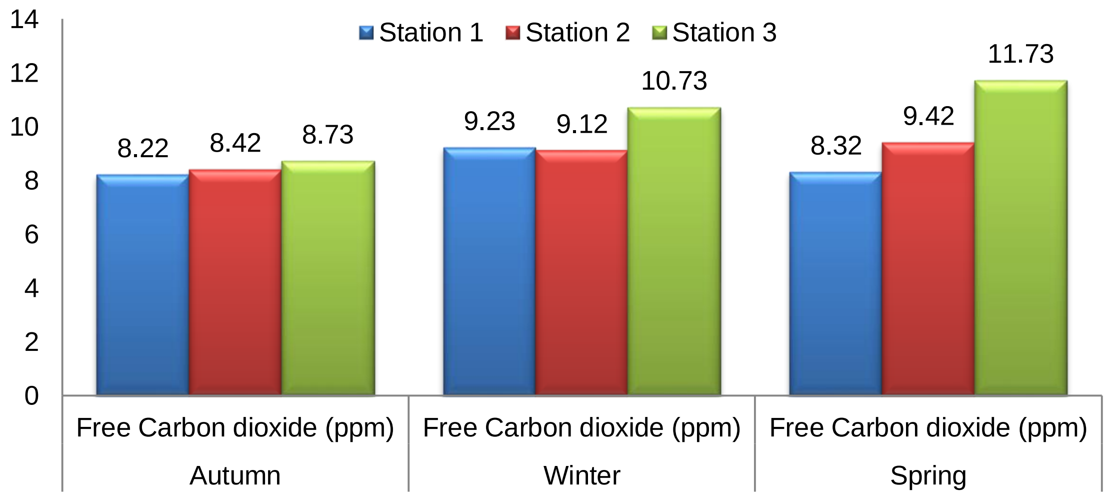

Figure 9: Variation of free Carbon dioxide at different seasons

4.3. Correlation between the environmental variables and fish species.

4.3.1. DCA (Detrended correspondence analysis with 26 segments.)

The detrended corresponding values indicated that there is strong correlation between the environmental variables and fish diversity (Table 2). The value of axis length was calculated to be between 2-4 which indicated that further analysis has to be done by using multi-variant tool i.e. CCA for more fruitful results.

Table 2: Values of DCA with their axes lengths.

	DCA1	DCA2	DCA3	DCA4
Eigenvalues	**0.0606**	**0.0213**	**0.0052**	**0.0009**
Decorana values	**0.0521**	**0.0212**	**0.0042**	**0.0019**
Axis lengths	2.4643	**2.1133**	**1.9223**	**2.2203**

4.3.2. Canonical Correspondence Analysis (CCA)

In the correlation of abundance of fish species towards different environmental variables were different and the positioning the 26 fish species in relation to environment variable is shown in fig 10. There is strong influence of dissolved oxygen and velocity basically in station 1 and station 2 in spring and winter seasons respectively where cluster of fishes like *Labeo boga* (C20), *Acrossocheilus hexagonolepis* (C15), *Puntius sophore* (C26), *Mystus vittatus* (C22) and *Xenentodon cancila* (C1) were found to be strongly correlated with those parameters. Similarly, autumn seasons stations 2 and 3 and spring season station 3 was found to be more favourable for the distribution of fishes like *Puntius ticto* (C2), *Barilius vagra* (C5), *Chana gachua* (C3), *Brachydanio rareo* (C13), *Labeo dero* (C19), *Wallago attu* (C24) and *Schistura devdevi* (C25). Furthermore, species like *Chana punctatus* (C6), *Esomus danricus (*C6), *Macrognathus pancalus (*C7*)*, *Tetraodon cutcutia (*C8*) Barilius barila (*C12), *Mystus bleekeri* (C14), *Oreochromis mossambicus* (C17) *Glyplothorax alkanandi* (C21*) and Mystus tengara* (23*)*, showed positive correlation towards temperature. On the contrary, species like *Lepidocephalichthys guntea* (C10), *Chela laubuca* (C9), *Anabas testudienus* (C11) and *Opsarius tileo* (C16) showed negative correlation towards free CO_2 as shown in the figure 10.

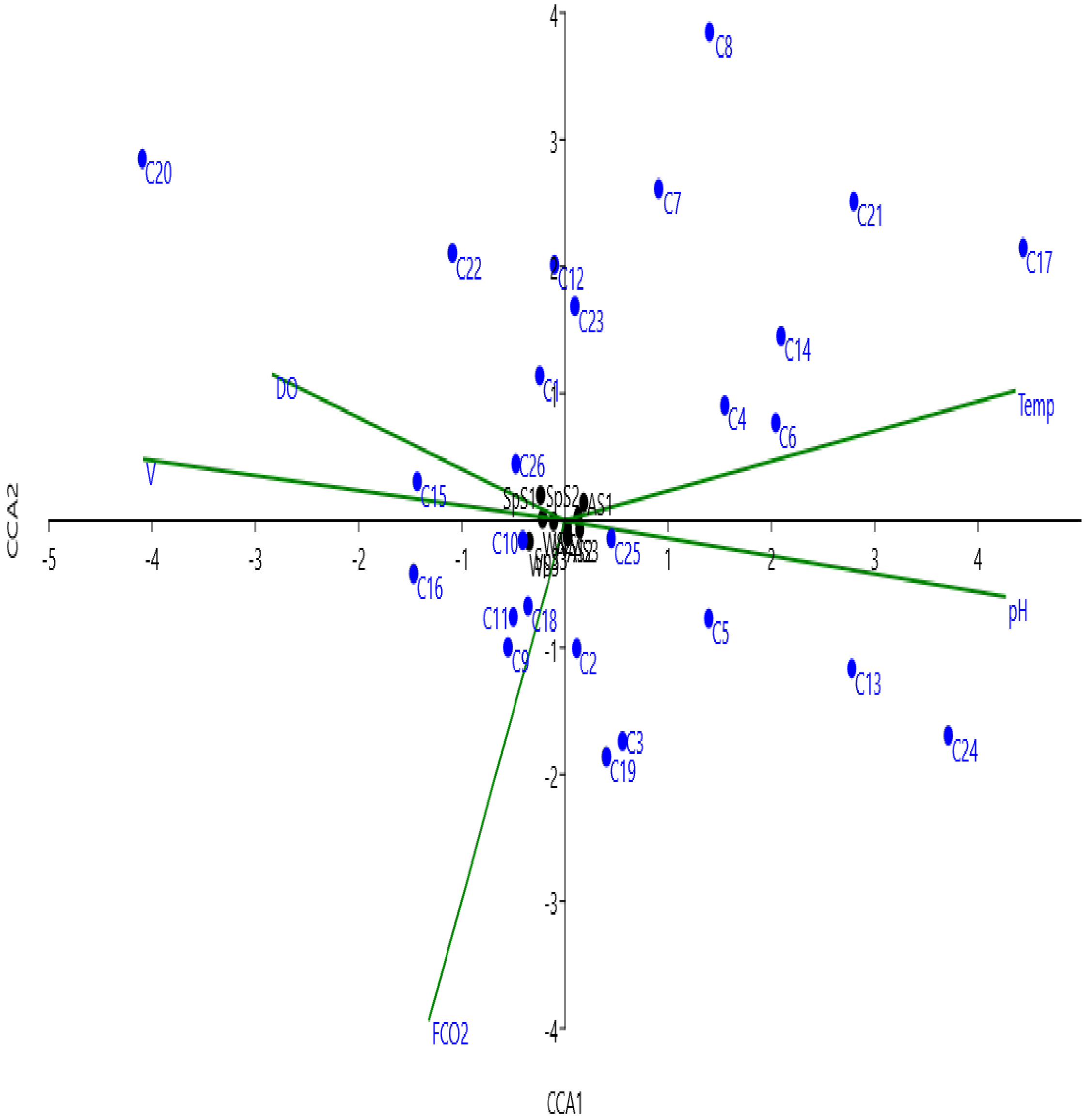

Figure 10: CCA plot of environmental variables Vs Fish diversity.

5. Discussion

The research explored the diversity of fish in relation to the water quality of the Dhungre River. Total, 2200 fish individuals were collected from the study, which included 26 species of fish from 7 orders, 11 families, and 20 genera. This indicated that Dhungre River supports a major source of livelihood and food to those fisherman and fishing communities depending upon fishery practice. This study differs with findings of (Limbu, et al. 2018; Hossain et al. 2018) according to which abundance of fish is high in month of February (spring season) and gradually decreases with rise in summer. The present study accounts the number of fishes in different seasons were supporting the argument of seasonal variation of fish distribution of Dhungre River. Eigen values of CCA for the first four axes CCA1, CCA2, CCA3 and CCA4 were found 0.29, 0.27, 0.08, and 0.07, respectively which was much lesser in this study that is CCA1 0.05, CCA2 0.02, CCA3 0.01, and CCA4 0.009 (Dudgeon D, et al. 2006) . This difference in eigen value could possibly be because the vast differences between the estuaries salinity and freshwater river without salinity. DO values ranged from 6.2 to 7.18 ppm almost same value reported by (Shrestha 2019) in similar river like Dhungre. Minimum values of DO were recorded during autumn season and maximum during spring months. Minimum DO in autumn months may be due to high metabolic rate of organisms. Though variation in DO concentration in Dhungre River is not so high, but (Kamal 1992) also observed variation in DO content of Moheskhali channel water. As per (Ahammad 2004), the Moheskhali channel's DO content ranged from 3.63 to 6.83 ppm. This may be due to high lapse of time of the both studies gave time for human industrial and chemical factories escalation and randomness in waste management systems polluted the water systems. pH value of the present study shows the similarity with (Dutta et al. 1954), (Martin 1970), (Adhikari 2019), (Noori 1999), (Belaluzzaman 1995) and (Rshid 1999). High pH value at station 2 is due to presence of bridge and extreme human interference with domestic waste disposal. Minimum water temperature was recorded at station 1 and maximum at station 2 and 3 according to seasonal changes. Adhikari (2019) and Thirumala et al. (2011) also reported variation of water temperature for seasonal change.

Though temporal variation in chemical parameters was observed due to seasonal variation but no spatial difference was found in this study. A total of 13 Cypriniformes fish species were recorded during the study period. Among them *Labeo dero, Labeo boga, Puntius sophore P. ticto and Esomus danricus* are each contributing more than 50% of the composition. Adhikari (2019) reported about 33 species among them 24 (65%) species Cypriniformes was collected by different types of net from Mechi River where (Jha 2014) observed 108 species from the Narayani River out of which 49% composition was contributed by Cypriniformes. These similarities in collections of Cypriniformes species are due to mostly identical seasons throughout Tarai reagion of Nepal. As present study area is the smaller riverine system in Nepal, findings showed a small number of species composition compared to Naryani river study of 2019 and 1991 although more or less similarities was found with Indreni, Mechi, Pampa, Bagmati, Lothar and Tanai rivers. The result of this study showed that most species of fishes were found to be abundant during autumn season and in station 3 which has approach to both warm and cold water fishes. Hence, the diversity index at station 3 was found to be highest among all where the value of H was found to be 2.66 followed by D = 0.85, and j = 0.38. It meant that the fishes at station 3 were highly abundant during all three seasons and were evenly distributed as well. The result contradicts with the findings of (Keskin and Unsal 1998). The possible reason could be the availability of sufficient volume of water which is comparatively untouched as compared with other sites. It might be due to availability of sufficient dissolved oxygen and favourable temperature. Besides this station is cool during winter and can adjust many cold water fish species and in winter and spring the water over here becomes slightly warmer which allows most warm water fishes to adjust at the same station.

Several pools, rifles, and run habitats arise throughout the winter. This pattern produced by variation in flow, also appeared to affect the physico-chemical variation in the lower streams. This could be the reason of assemblage many small indigenous species like *Olyra longicaudata*, *Anabas spp and Channa spp.* A homogenous pattern of physico-chemical variation was reported by (Closs and Lake 1994) in a stream in Australia. They showed the low flow period created a filter that was responsible for structuring the fish and aquatic ecosystem of the River. Physical parameter variation in fish communities has been reported to be influenced by variation in the velocity, stream size and covariate of these factors such as temperature and pH (Meffe and Sheldon 1988). In the same way the physical parameters pattern was observed in the fish community of Dhungre River showed same result with highest rank of Shannon’s index during the autumn season. This was also shown by low variability in the DCA values and by the strong relationship of fish assemblage with the environmental variables shown by the CCA plot. According to the results achieved during this study it makes explicit that the fish Dhungre River locating in the mid southern part of

Nepal is rich in fish fauna along with other aquatic components. Considering that the research field work was carried out for three sequential seasons i.e. autumn, winter and spring season among which the species contingency was found to be upmost in the autumn season. Though it showed high diversity of fishes in the autumn season most fishes were thriving the seasonal patterns according to their adaptational features.

The outcomes comprehended that various physico-chemical parameters like DO, CO_2, temperature and velocity were performing the crucial role in fish diversity and evenness and beside these other elements like pH, transparency were vital in micro-level for fish dispersal. Thus the fishes like *Chana punctatus, Esomus danricus, Macrognathus pancalus, Tetraodon cutcutia, Barilius barila, Mystus bleekeri* and *Oreochromis mossambicus* were found at higher temperature zones in this study.

A biodiversity index attempts to characterize the diversity of a sample or population using a single number (Magurran 1988). The idea of ‘‘species diversity" has two components: the number of species or richness and the distribution of individuals among species. The formal handling of the idea and its measurement, on the other hand, is complicated (Williamson 1973). Shannon’s diversity index considers the richness and proportion of each species while Evenness and Dominance indices represent the relative number of individuals in the sample and the fraction of common species respectively. The biodiversity index values (H’) obtained from present study is not so very high according to Shannon’s diversity index values and they do not exactly show the differences occurring among the stations either. The explanation for the decreased species richness, according to (Keskin and Unsal 1998), is that the fishing gears utilized have a high selectivity impact. The equipment effect of the fishing gear used in this study was not investigated due to lack of resources. Despite this, the fact that the fishing gear showed lesser selectivity during sampling offers an indication about the fish species richness in the region and demonstrates the presence of a medium-sized biodiversity in the region. Highest Shannon diversity index was found in autumn season and at station 1 where lowest was observed at station 3 and during winter. In each scenario, a high Shannon diversity index is associated with a small percentage of individuals, whereas a low diversity index is associated with a large number of individuals. The primary reasons of changes in biodiversity indices are seasonal fluctuations in nutrients at sea grass beds, which impact the cohabitation of many fish species (Huh and Kitting, 1985), atmospheric air currents and environmental conditions (Keskin and Unsal, 1998), and seasonal fish migrations (Ryer and Orth 1987). Samples from first season months

(September–November) maintained a similarity in counts at all stations which is also same for last three months (March–May). Station 1 and 3 showed some dissimilarity with station 2 because this area was buffer zoned to minimize the over exploitation of resources by Chitwan National Park committee.

6. Conclusions

Altogether 2200 fishes were counted out of which 26 species of fish belonging to 7 orders, 11 families and 20 genera were classified throughout the three seasons. *Puntius sophore* was found to be most abundant fish species while *Oreochromis mossambicus* least abundant during the study period. The fishes of Dhungre River were undoubtedly relies upon freshwater diversity and evenly distributed except few species were prone to some different types of habitat and behaviour. Comprehending the correlation between fish diversity and environmental factors could better explained by physico-chemical patterns in the fish diversity. The upshots in the current research conveyed that the seasonal variations in the fish diversity were largely related to water parameters like DO, temperature, velocity, free carbon dioxide, and the pH of the water. Moreover, single diversity index could not utterly speak of the diversity as each diversity component had different environmental effects. So the current study has tried to employ a variety of other diversity indices to reconnoitre the water quality and seasonal patterns in fish diversity. Finally, the current study might be the most important research work for future studies of the Dhungre River and related water flora and fauna throughout futuristic research as long as it remains unbiased.

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APPENDIX

Table showing fish distribution in Dhungre River

Table 3: Distribution of fishes according to season and stations

Scientific Name	Autumn			Winter			Spring			Total
	Station 1	Station 2	Station 3	Station 1	Station 2	Station 3	Station 1	Station 2	Station 3	
Xenentodon cancila	25	12	19	12	9	10	14	14	11	116
Puntius ticto	44	66	70	22	10	32	30	32	36	342
Barilius vagra	12	2	6	5	5	2	2	3	10	47
Chana punctatus	6	4	8	0	2	1	4	8	6	39
Chana gachua	7	0	3	3	3	2	1	11	10	40
Esomus danricus	15	22	28	4	2	1	8	12	7	99
Macrognathus pancalus	4	2	1	0	0	1	2	2	1	13
Tetraodon cutcutia	2	0	1	0	1	0	1	2	1	8
Chela laubuca	3	1	3	1	0	2	3	2	4	19
Lepidocephalichthys guntea	38	50	28	19	15	13	22	2	14	201
Anabas testudienus	20	9	11	9	13	7	11	11	21	111
Barilius barila	19	18	4	4	11	2	13	10	9	90
Brachydanio rareo	6	15	19	2	2	0	0	2	1	47
Mystus bleekeri	9	2	2	0	0	1	2	1	2	19
Acrossochellus hexagonolepis	2	4	0	0	1	2	2	2	1	14
Opsarius tileo	13	27	24	5	15	21	22	21	18	166
Oreochromis mossambicus	1	0	0	0	0	0	0	2	1	4
Olyra longicaudata	10	21	31	2	12	12	15	18	17	138
Labeo dero	2	3	5	0	1	1	2	1	3	18
Labeo boga	2	2	0	1	0	2	3	0	0	10
Glyplothorax alkanandi	8	2	3	0	0	1	1	1	0	16
Mystus vittatus	2	1	11	4	0	0	12	2	8	40
Mystus tengara	2	2	3	3	0	0	2	0	1	13
Wallago attu	1	1	3	0	0	0	0	1	1	7
Schistura devdevi	31	12	34	12	12	7	25	28	42	203
Puntius sophore	40	52	88	21	35	27	44	34	29	370
									Total	2200

Chart showing different physical and chemical parameters of Dhungre River:

Table 4: Physico-chemical parameters of Dhungre River at various stations and in different seasons

S.N.	Parameters	Autumn			Winter			Spring		
		Station 1	Station 2	Station 3	Station 1	Station 2	Station 3	Station 1	Station 2	Station 3
1	Temperature (‘c)	20	21	21	15	16	15	19	21	20
2	pH	7.48	7.63	7.54	7.12	7.23	7.24	7.28	7.63	7.54
3	Velocity (m/s)	0.25	0.52	0.46	0.29	0.62	0.86	1.1	0.41	0.56
4	DO (ppm)	6.15	5.82	6.43	7.22	7.56	8.1	9.6	8.54	8.2
5	Free CO2 (ppm)	8.22	8.42	8.73	9.23	9.12	10.73	8.32	9.42	11.73

PHOTOPLATES

Fish sampling station 1, Dhungre Khola

Fish sample preserved for further identification Fish sampling station 2, Dhungre khola

Chana punctatus

Chana gachua

Xenentodon cancila

Barilius vagra

Puntius ticto

Mystus tengara

Mystus vittaus

Esomus danricus