Fish community structure along altitudinal gradients with relation to environmental variables in Ratuwa River of Eastern, Nepal

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Abstract
Studies on fish community structure along altitudinal gradients of rivers are lacking in Nepal. This study was carried out to gauge the fish diversity and composition along elevational gradient in Ratuwa River. The altitudinal gradient varies between 70 m to 1300 m. Fish samples were collected based on habitat representativeness from April (spring) 10-18, July (summer) 10-18, October (autumn) 10-18, 2020 and January (winter) 10-18, 2021. A total of 3447 specimens representing 4 orders, 14 families and 36 species were identified. Both fish diversity and abundance of studied ichthyofauna vary with altitudinal gradient. The present study affirmed that fish species of Opsarius bendelisis, Schistura multifasciatus, Garra annandalei, Brachydanio rerio, Aspidoparia morar, and Schistura scaturigina are the major contributory species (>1%) for both space and time spectrums. Fish community structure testing for both to space and time showed significant difference in spatial spectrum (R=0.72, P<0.01) but no significant difference in temporal variation (R=-0.034, P>0.05). The present results hinted that fish assemblage structure varied significantly from low to high elevations, altitude, water temperature, water velocity, dissolved oxygen and pH was found as major influential factors (P<0.05) for species distribution.

Key words: Altitude, freshwater, fish diversity, stream

Introduction
Studies on fish community structure of freshwater fishes along the elevation gradient of rivers and streams are scanty in Nepal (Limbu and Prasad, 2020; Limbu et al., 2020; Prasad et al., 2020) and also studies on fish diversity in relation with environmental parameters and habitat, spatially and temporally aspects are very less (Limbu et al., 2021). Countless studies have been conveyed out on altitudinal models of species richness in mammals, birds and plants; but hardly any studies have been done in fish (Bhatt et al., 2012; Carvajal-Quintero et al., 2015). The falling off in species richness and extension in taxonomic
distinctiveness with increasing elevation is a well-archived circumstance in the flora and fauna (Rahbek, 1995; Lomolino, 2001). Broad land surface area is anticipated to assist more species and individuals under similar climatic conditions (Rahbek, 1995; Sanders and Rahbek, 2011). Biological interactions such as competition, predation, and productivity can control the occurrence of species and, to a greater extent, species richness (Whittaker, 2010; Stein et al., 2014). Broadly, the noticed model is blemished by lessen in species diversity and the substitution of species as elevation increases; however, the processes that determine those patterns to be left poorly observed (Lomolino, 2001).

Physical and chemical characteristics factors are imperative determinants of the condition of the fish species richness, abundance and distribution. For instance, habitat variables, such as substrate structure (Edds, 1986; Merz and Ochikubo Chan, 2005), flow rate and water velocity (Edds, 1986; Yu and Lee, 2002; Nelson and Lieberman, 2002), temperature (Vought et al., 1998; Kadye et al., 2008), stream size and altitude (Magalhaes et al., 2002; Gerhard et al., 2004) have been widely proved to influence fish community structure. Numerous studies (Edds, 1986; Mishra and Baniya, 2016; Pokharel et al., 2018; Limbu et al., 2019a, 2019b, 2020) have demonstrated the retaliation of different taxa along environmental variables in rivers. But, the review of literature shows that the study of fish diversity with relation to elevation, environmental variables and fish habitat aspects at different space and time scales are yet to be done.

There have been few studies that have described the patterns of ichthyofauna diversity along altitudinal gradients. It is critical to gain a better understanding of the diversity patterns of freshwater fish fauna across altitudinal gradients in order to determine how anthropogenic activities affect these patterns (e.g. hydroelectric dams, the introduction of exotic species, agriculture, deforestation and pollution). This data is necessary for developing successful conservation measures and mitigating the effects of human activity. Therefore, the aims of the study were to determine whether stream fish diversity varies with altitude and to evaluate which azoic element govern species richness and configuration.

Materials and Methods

Study Area

The present study was conducted in the Ratuwa River and is situated in the part at Mangsebung Rural Municipality in Ilam district (Figure 1). The river originates from the Chure range at latitude (26.656°) and longitude (87.705°) and altitude (1853 m). River gradient decreases as the river surge into the relatively flat region in the lower Terai reaches (1853 m-70 m). The river substrata originate mostly erosion and weathering of sandstones in rainy season and can be characterized as fine to coarse sand, gravel, cobbles, pebbles and boulders. Alnus nepalensis is major natural vegetation for gradient (600 m-1853 m) and river bed is characterized as mostly big boulders, cobbles, pebbles and little sand. In contrary, Shorea robusta, Schima wallichii and the bushes are major natural vegetation for gradient (600 m-70 m) and river bed mostly consists of sand, cobbles, pebbles and gravels. From origin point to the few kilometers, river seems to be just like a canal and harbors no fish. It surges about 45 km towards the south and shows linkage with many canals and tributaries and finally discharges into Bay of Bengal, India. The river is blessed with both tropical and temperate climate which enable to advocate a wide biological diversity.
Data Collection

In this work, the altitudinal gradient of the studied sites varies between 70 m - 1300 m. We allocated altitudes into two altitudinal zones; STA: 70 m – 600 m and STB: 600 m – 1300 m (ST5, ST6, ST7 and ST8) for hydrological parameters and fish collection. Furthermore, fish samples were collected based on habitat representativeness from April (spring) 10-18, July (summer) 10-18, October (autumn) 10-18, 2020 and January (winter) 10-18, 2021. Based on the characteristics of the flow pattern, slope, average velocity and substrata components in the study area (Han, 2010; Huang et al., 2019; Limbu et al., 2021), the habitat types were categorized into three groups: lentic habitat (pool and steep pool), slow flow pattern (glide and run) and fast flow (riffle and cascade). Each sampling site was 200-250 m long with different habitat types (for instance, pool, steep pool, glide, run, riffle and cascade). For the fish sampling, two cast nets of different sizes were used, one having small mesh size of 0.5 cm, 3 m diameter and 2 kg weight and
another having large mesh size of 2 cm, 6 m diameter and 6 kg weight, with the help of local fisherman. For the laboratory examination, about 10% collected fish were preserved in 10% formaldehyde solution in plastic jar by making their head upside for the protection of their caudal fin and after the photography, remaining samples were returned to their own natural habitat from where they were captured. The identification was done with the help of standard taxonomic references (Talwar and Jhingran, 1991; Jayaram, 2010; Shrestha, 2019; Fricke et al., 2021).

Water temperature (°C), dissolved oxygen (DO) (mg/l), water pH, and water velocity were measured in-situ by a standard method (APHA, 2012), using a digital thermometer by placing it in the water at a depth of 1 feet for two to three minutes, DO meter (Model: DO5509, Lutron), pH meter (HI 98107, HANNA Instruments), whereas water velocity was measured by the float method with the help of a stop watch, plastic ball and measuring tape.

Data Analysis
To highlight the differences in environmental parameters (i.e, pH, water temperature, dissolved oxygen and water velocity) and fish community attributes (i.e, Shannon index, Evenness index and Dominance index) between the different months, sites and altitude we executed analysis of variance (ANOVA) using the function aov in R software (R core Team, Vienna, Austria). In the event of significance, a post hoc Tukey HSD test was used to determine which means were significantly different at a 0.05 level of probability (Spjotvoll and Stoline, 1973). One-way analysis of similarities (ANOSIM) (Clarke, 1993) was used to test the significant difference among the spatial and temporal scales. To visualize the major contributing species both to space and time, similarity percentage (SIMPER) (Clarke, 1993) analysis was performed. Of 36 fish species, 6 species occurred in <5% of the samples and removed from the analysis followed by Gauch (1982), who pointed out most of the multivariate analysis are influenced by rare species and are also outliers, thus obscuring the analysis of the data set as a whole. To verify the relationship between a spatial or temporal ordination of fish species and water parameters, the abundance matrix was pulled to Detrended Correspondence Analysis (DCA) and the gradient length was estimated. As the gradient length obtained from DCA was long 5.67 indicating Canonical Correspondence Analysis (CCA) was more applicable to verify the existence of fish species and azoic factors.

Results
A total of 3447 specimens representing 4 orders, 14 families and 36 species were collected (Table 1). The ichthyofauna comprised mainly of Cypriniformes (22 species) followed by Siluriformes (7 species), Synbranchiformes (3 species) and Anabantiformes (4 species). Total number of fish at each site varied from 1 to 594 individuals. Results from the similarity percentage analysis (SIMPER) showed that 24 species contributed more than 1%. Of these, Barilius bendelisis, Schistura multifasciatus, Garra annandalei, Brachydanio rerio, Aspidoparia morar, and Schistura scaturigina were the abundant species both to spatial and temporal spectrums. One-way analysis of similarity (ANOSIM) testing for both spatial and temporal variations in fish community suggested that there was a significant difference in spatial spectrum (R=0.72, P<0.01) but no significant difference in temporal variation (R=-0.034, P>0.05). Species richness sequentially increased from station (ST1-ST3) but decreased in station (ST4).

<table>
<thead>
<tr>
<th>Order</th>
<th>Family</th>
<th>Code</th>
<th>Species</th>
</tr>
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<tbody>
<tr>
<td>Cypriniformes</td>
<td>Cyprinidae</td>
<td>Sp1</td>
<td>Labeo bata Day, 1877</td>
</tr>
<tr>
<td></td>
<td>Cyprinidae</td>
<td>Sp2</td>
<td>Garra annandalei Hora, 1921</td>
</tr>
<tr>
<td></td>
<td>Cyprinidae</td>
<td>Sp3</td>
<td>Puntius terio Hamilton-Buchanan, 1822</td>
</tr>
<tr>
<td></td>
<td>Cyprinidae</td>
<td>Sp4</td>
<td>Puntius sophore Hamilton-Buchanan, 1822</td>
</tr>
<tr>
<td></td>
<td>Cyprinidae</td>
<td>Sp5</td>
<td>Puntius conchonius Hamilton-Buchanan, 1822</td>
</tr>
</tbody>
</table>

Table 1. Fish species collected from Ratuwa River.
Altitude had a significant effect on the fish community parameters. Species richness was negatively related to elevation ($r = -0.67$) and species richness per site ranged from 5 to 22 species (Table 2), whereas there was a positive correlation between Shannon-Weiner diversity index and dominance index ($r = 0.88$). In contrary, both Shannon-Weiner diversity index and Dominance index were negatively related to elevation ($r = -0.56$ and $r = -0.33$). Highest number of individuals was found at elevation between 71 m to 200 m with decrease at higher elevations (Fig. 2).

<table>
<thead>
<tr>
<th>Table 2. Showing spatial abundance and richness of fish species in different stations and different altitude.</th>
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<tbody>
<tr>
<td>Station</td>
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(a) Species richness vs. Altitude
(b) Dominance (D) vs. Shannon (H)
The first and second axis of canonical correspondence analysis (CCA) explained 53% of the total variance (37% on the first axis and 16% on the second axis). The CCA biplot indicated the relationship between fish species and environmental variables (Figure 3). The first axis obtained from CCA explained a gradient associated with water velocity, pH and water temperature. The fish species associated with these environmental factors (water velocity and pH) were sp1, sp5, sp8, sp10, sp29, sp30 and sp31. On the contrary, fish species, sp4, sp7, sp9, sp11, sp13, sp14, sp21, sp22, sp23, sp24, sp25, and sp28 were highly associated with water temperature. While the second axis explained the variables of dissolved oxygen (DO) and altitude. Fish species, sp2, sp12, sp15, sp16, sp18 and sp21 were highly associated altitude. On the other hand, species of sp6, sp17, sp19 and sp20 were linked with dissolved oxygen. Analysis of CCA indicated that all the selected environmental

Figure 2. Regression models between species richness (a), Shannon-Weiner H vs dominance index, D; (b), Shannon H vs Dominance D; (c) Altitude vs Shannon H; (d), Altitude vs Dominance D; (e), Altitude vs Abundance, abundance and the elevation gradient in the Ratuwa River.
factors greatly influence ($F=5.41$, $P<0.05$) the fish community structure in Ratuwa River.

**Figure 3.** Biplots from canonical correspondence analysis (CCA) for the fish community structure in Ratuwa River (do= dissolved oxygen, wt= water temperature, wv= water velocity; for species code please see Table 1)

**Discussion**

The present study examined ichthyo-fauna community structure along elevation gradients in Ratuwa River of eastern Nepal. Our results hinted that fish assemblage structure varied significantly from low to high elevations. This is due to unalike fish species distribution and composition, which can be affected by environmental variables such as elevation, area of elevation band (Cruz-Elizalde *et al.*, 2016), and productivity (Wright *et al.*, 2003). Our study is also in agreement with studies of fish species in the eastern Europe (Askeyev *et al.*, 2017), stream of Northen east Algeri (Benzina *et al.*, 2019), Yangtze River basin (Fu *et al.*, 2004), in the southern Appalachians (Robinson and Rand, 2005), in eastern Nepal Himalaya (Khatiwada *et al.*, 2019), in Himalaya (Bhatt *et al.*, 2012), and in the central Andes (Jaramillo-Villa *et al.*, 2010), which have described that the diversity of aquatic fauna decreased gradually with altitude. The species richness or diversity in the uplands lessened significantly along the altitudinal gradient, which hints that the environmental factors of the high land or altitude might serve as a barrier to scatter and the colonization of adjacent drainage basins by lowland fishes (Pouilly *et al.*, 2006; Jaramillo-Villa *et al.*, 2010).

Our multivariate analysis indicated that altitude, dissolved oxygen, water velocity, water temperature and pH were significant ($F=5.41$, $P<0.05$) contributors in shaping the species distribution. Water temperature (Kadye *et al.*, 2008; Limbu *et al.*, 2021) and dissolved oxygen (Pouilly *et al.*, 2010; Limbu *et al.*, 2019) have already been shown to affect the fish community. Besides, current velocity (Yu and Lee, 2002; Li *et al.*, 2012), depth (Vlach *et al.*, 2005; Kadye *et al.*, 2008), width (Gerhard *et al.*, 2004), substrate (Vlach *et al.*, 2005; Limbu *et al.*, 2020), altitude (Magalhaes *et al.*, 2002; Bhatta *et al.*, 2012), conductivity (Yu and Lee, 2002) and climate (Magalhaes *et al.*, 2002) have all been shown to influence fish species distribution. Fish species, *Schistura multifasciata*, *S. sovana*, *S. horai*, *S. scaturigina*, *S. rupecula* and *Schozothrax plagiostomus* showed positive response to elevation. Only those
species which has been well adapted to highland, rapid flowing habitats and exhibit number of morphological modifications in their lips, body shape and size and also other associated structures and color patterns (Wang et al., 2006). This study demonstrated that the acknowledgement of the distinctive upstream ichthyofauna to altitudinal gradient range diverse from that of species populating downstream rivers.

Our present results suggested that the Shannon index decreased with elevation. The fish diversity of common of typical species in the community decreased on an elevational gradient (Askeyev et al., 2017). Similarly, Simpson’s index also did the same as Shannon but did not support the previous studies (Jaramillo et al., 2010; Askeyev et al., 2017). Only Schistura spp were dominated above 700 m. This can be explained of the fish abundances to the existing available natural resources (Matthews, 1998; Askeyev et al., 2017). Fish diversity and total abundance showed unimodal response to elevation (Askeyev et al., 2017). The highest fish numbers were recorded at elevation between 71 and 200 m. Our study suggested that the species richness and abundance increased with decreased elevation. A similar pattern has been reported elsewhere water bodies (Rahbek, 1995; Lomolino, 2001; Bhatta et al., 2012; Askeyev et al., 2017). According to those studies, species richness in the upstream damened significantly, with a reduction of 3.3–17.0% of the total number of species present along the altitudinal gradient. The higher species richness and abundance of fish increased could be influenced by many factors such as sufficient amount of food availability, water velocity, volume, width, depth, vegetation and topography.

Our analysis of similarity (ANOSIM) testing for both spatio-temporal variations in fish community suggested that there was a significant difference in spatial spectrum (R=0.72, P<0.01) but no significant difference in temporal variation (R=-0.034, P>0.05). This type of spatio-temporal spectrum of variation in ichthyofaunal diversity observed in our study is also found in other river systems (Yan et al., 2010; Li et al., 2012; Limbu et al., 2020; Shrestha et al., 2020; Adhikari et al., 2021; Limbu et al., 2021). In addition, the extremely low R value suggested that seasons are not major factors to influence the species variation but the spatial spectrum significantly changed the species variation.

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