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Spatio-temporal variation of fish assemblages in Babai River of Dang district, Province No. 5, Nepal

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Abstract

Spatial and temporal variation of fish assemblages were investigated seasonally from October 2018 to May 2019. Fish assemblages were agglomerated with environmental variables both to spatial and temporal scales. Water temperature, dissolved Oygen, free carbon-dioxide, pH and water velocity of water of each site were measured. Based on analysis of similarities (ANOSIM), fish assemblages were significantly different in spatial variation but not in temporal variation. A total of 1,024 individuals belonging to 5 orders, 9 families and 15 genera and 24 species were collected. The dominated species were *Puntius sophore*, followed by *P. terio*, *P. ticto* and *Barilius bendelisis.* The Redundancy Analysis (RDA) vindicated that environmental variables of water temperature, pH, water velocity and free carbon-dioxide were found to be contributed variables to shape the fish assemblage structure of Babai River. The cluster analysis delineated that similarity between fish species decreases as the distance of sites increased.

Keywords: Babai River, Cluster, Fish Diversity, RDA, Spatio-Temporal Pattern

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Introduction

The diversity of the natural population is partially dependent on the environmental variables which always affect the competing populations (Chowdhury *et al.,* 2010; Hossain *et al.,* 2012). The factors influencing fish assemblages involve the environmental variables which are spatially heterogenous and temporally variable and biotic interactions such as competition and predation (Gorman, 1988; Harvey and Stewart, 1991; Grossman *et al.,* 1998). Most important environmental variables are temperature, dissolved oxygen, pH, depth, free carbon-dioxide, total hardness, and water velocity (Yu and Lee, 2002, Pessanha and Araujo, 2003, Kadye *et al.,* 2008, Limbu *et al.,* (2019a) and fisheries diversity of river are also affected by chlorophyll-a, altitude, conductivity substrata, distance to source, and climate (Blanc *et al.,* 2001; Magalhaes et al., 2002; Yu and Lee, 2002). However, changing environmental parameters can affect biotic communities in multiple ways and function of ecosystems (McGill *et al.,* 2006; Conversi *et al.,* 2015). Environmental variables are reported to shape the spatial distribution of species (Perry *et al.,* 2005) and influence the temporal variation of communities (Rouyer *et al.,* 2008).

Fish assemblage structure in the rivers and streams of Nepal has not been well studied. The spatial and temporal variations of the fish assemblages in rivers and streams of Nepal are poorly understood (Limbu *et al.,* 2019b) and still thousands of rivers, streams and lakes are being unexplored (Limbu *et al.,* 2018). To the best of our knowledge, there is no any report of fish diversity of Babai River. So, to overcome above research gaps we have initiated the present research to determine the spatio-temporal

variation of fish assemblages of Babai River, Dang, Nepal and the physico-chemical variation of their aquatic habitats.

The present study area, Babai River is a medium sized perennial river and situated in Dang district of Province no. 5 which originates from the eastern end of Dang valley and surges through the Laxmipur, Saudiyar, Duruwa, and Urahari before meeting its tributary Sharada River at Kalimati Kalche and flows through the lowland

Study area

of Dang, Banke and Bardiya district. The study area is located in the Dang district which starts from Urahari to Motipur at along the Babai River. The four stations (Fig. 1) were taken at Urahari, Shitalpur, Phulbari and Motipur within the length of 7 km.

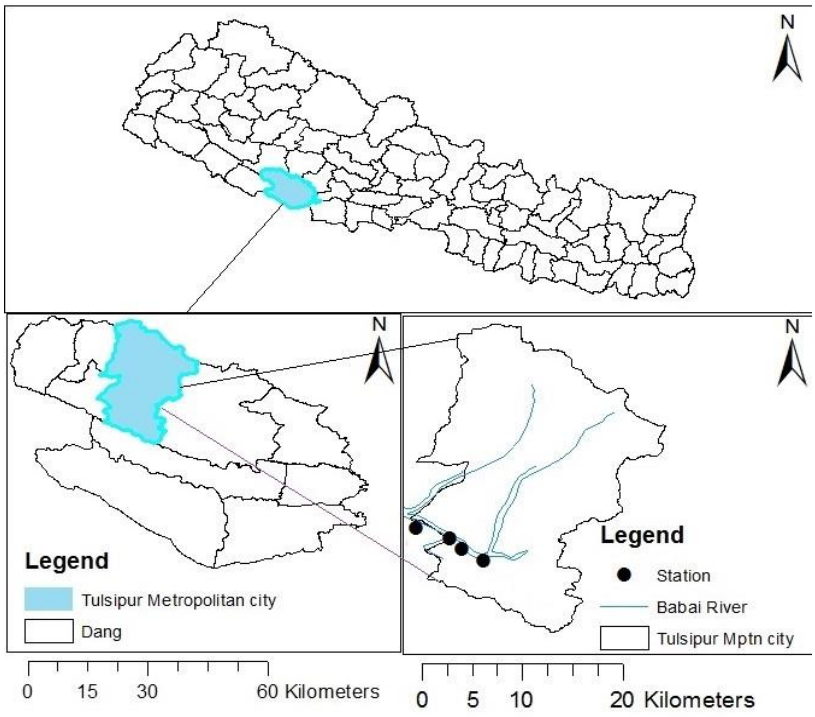


Figure 1. Map of study area of Babai River.

Sampling sites

Altogether four sampling sites (A, B, C, D) were chosen along the River side. Sampling sites were selected on the basis of human settlement area, confluence point of river and near the Tulsipur-Amelia Highway (Fig. 1) The sampling site A (28º01'54.2″N and 82º16'19.3″E) was selected at the conflux point of Sewa khola and Babai, in Urahari. The sampling site B (28º02'28.7″N and 82º15'10.8″E) was chosen near the bridge of Tulsipur- Amelia Highway in Shitalpur. This area is where people use as picnic spot and of religious value. The River bed consists of cobble, pebble and sand. The third sampling site C (28º02'52.2″N and 82º14'15.03″E) was selected at Phulbari which is the broader part of the River. The River bed consists of cobble, pebble and sand. The sampling site D (28º03'38.1″N and 82º13'8.6″E) was selected at Motipur near Forest area. This site is the area after the conflux point of Tui Khola.

Sampling time period

The field work was conducted from October 2018 to May 2019. Each sampling site was visited in the month of October (Autumn), January (Winter) and May (Spring).

Materials and methods

For the fish agglomeration cast net, Bamboo fish trap, Hapa net, and mosquito nets were used. Cast net of 6 mm × 6 mm mesh size was used for the collection of fishes. Fish sampling was done at 7 am to 11am in each station with the help of local fisherman. Before preservation, collected fishes were photographed with Nikon Digital Camera. After photography, the collected fishes were preserved in 37% formalin solution for 6 to

8 hours and later, the fishes were preserved in 10% formalin solution making their head upside for the protection of their caudal fin for further study and 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 Jayaram (2010), and Talwar and Jhingram (1991).

The following environmental variables were analyzed during each field visit: water temperature, dissolved Oxygen (DO), pH, free CO_2 and water velocity. Water temperature (ºC) was measured with a digital thermometer by placing it in the water at a depth of 1feet for two to three minutes. DO (mg/l) was measured by the Winkler titra-metric method. Free CO_2 was measured by titration method. pH was measured by using a pH meter (HI 98107, HANNA Instrument). Water velocity was measured by the float method with the help of a stop watch, plastic ball and measuring tape.

Data analysis

Seasonal and site-wise Shannon-Weiner index, Simpson index (D), and Evenness index (J) were performed by using library "vegan" in R (Oksanen *et al.,* 2019). Fish species were analyzed into different assemblage clusters based upon abundances of each fish species by utilizing *pvclust* package in R (Suzuki and Shimoddaira, 2019). Samples by species, sites, seasons, and environmental variables were analyzed through multivariate analysis tool. At first, detrended correspondence analysis (DCA; Gauch, 1982) was performed to determine whether redundancy analysis (RDA), or canonical correspondence analysis (CCA) would be the most appropriate model to describe the association between species abundance, sites, seasons, years and environmental variables. The values of axis length and Eigen values obtained from DCA suggested that the linear model associated with RDA was more applicable. Therefore, a direct multivariate ordination method based on a linear response of species to environmental gradients (Gauch, 1982; Ter Break, 1986) was applied by using vegan library in "R" (Oksanen *et al.,* 2019). One way analysis of similarity (ANOSIM) (Clarke, 1993) was used to conclude the significance of spatial and temporal variation of fish assemblage structure.

Results

A total of 24 fish species belonging to 5 orders, 9 families and 15 genera were recorded from the total catch of 1024 fishes (Table 1, Fig. 8). *Puntius sophore* (180 individuals) with 17.40% of total catch was recorded as a dominated species while *Eresthestoides ascita* (1 individual) with 0.096% of total catch was the rarest species. *Puntius sophore, P. terio, P. ticto* and *Barilius bendelisis* were ubiquitously found both to time and space. Maximum number of individuals was recorded from site D with 332 catches and minimum number was recorded from site A with 144 catches. Seasonal variation and abundance of fishes increased in autumn and decreased in spring. Highest number of individuals (377) was recorded in autumn (February) and least number of individuals (299) was recorded in spring (May). The analysis of similarity (ANOSIM) showed significance difference in spatial variation (R=0.61, P<0.05) but no significance difference in temporal variation (R=-0.17, P>0.05).

Table 1. Fish distribution and frequency in Babai River, Dang

S.N.	Name of species	Autumn				Winter				Spring				Total	Frequency
		A	B	C	D	A	B	C	D	A	B	C	D		
1.	*Labeo fimbriatus (*C1)	-	-	1	1	-	2	3	2	-	-	-	-	9	0.87
2.	*Garra gotyla* (C2)	-	1	2	-	-	1	1	2	2	-	1	1	11	1.06
3.	*G. rupecula* (C3)	-	1	-	-	2	1	1	-	-	-	1	-	6	0.58
4.	*Puntius sophore (*C4)	12	39	16	20	11	4	8	12	-	11	14	33	180	17.40
5.	*P. terio* (C5)	10	17	14	19	5	-	7	9	-	4	15	18	118	11.41
6.	*P. chola* (C6)	5	9	7	13	1	4	11	-	9	-	8	5	72	6.96
7.	*P. ticto* (C7)	2	15	-	15	2	7	-	16	10	11	21	9	108	10.44
8.	*P. conchonius*	-	10	5	6	3	-	5	-	-	4	15	7	55	5.31

	(C8)														
9.	*Tor putitora* (C9)	1	-	-	-	-	-	-	1	-	-	-	-	2	0.19
10.	*Barilius bendelensis* (C10)	1	2	19	15	13	20	9	26	14	19	8	11	157	15.18
11.	*B. barila* (C11)	-	10	9	8	12	3	9	6	-	-	2	-	59	5.70
12.	*B. shacra* (C12)	-	-	-	-	5	8	15	9	-	-	-	-	37	3.57
13.	*B. modestus* (C13)	-	12	1	-	-	5	9	13	-	1	2	-	43	4.15
14	*B. barna (*C14)	-	2	-	-	-	3	1	-	-	-	1	-	7	0.67
15.	*Danio devario* (C15)	-	-	6	7	-	-	15	8	-	1	3	4	44	4.25
16.	*Psilorhynchus balitora* (C16)	-	-	2	-	1	-	1	3	2	-	-	1	10	0.96
17.	*Acanthocobotis botia* (C17)	1	1	12	6	2	2	3	10	3	6	3	5	54	5.22
18.	*Schistura savona* (C18)	-	-	-	1	1	-	-	-	-	2	-	-	4	0.38
19.	*Mystus bleekeri* (C19)	10	1	2	-	2	7	11	-	1	4	-	-	38	3.67
20.	*Erethistes pussilus* (C20)	-	1	-	-	-	-	-	-	-	-	-	-	1	0.096
21.	*Heteropneustes fossilis* (C21)	-	-	-	-	-	-	1	-	-	-	1	-	2	0.19
22.	*Xenentodon cancila* (C22)	-	2	-	-	-	1	-	3	1	-	1	-	8	0.77
23.	*Mastacembelus armatus* (C23)	-	1	-	4	-	-	-	-	-	-	1	1	7	0.67
24.	*Channa punctata* (C24)	-	-	-	-	-	-	-	-	-	-	-	2	2	0.19
	Total													1034	

Table 2. Fishes collected from Babai River.

SN	Order	Family	Genus	Species
1.	Cypriniformes	Cyprinidae	*Labeo*	*L. frimbitus*
			Garra	*G. gotyla*
				G. rupecula
			Puntius	*P. sophore*
				P. terio
				P. chola
				P. ticto
				P. conchonius
			Tor	*T. putitora*
			Barilius	*B. bendelensis*
				B. barila
				B. shacra
				B. modestus
				B. barna
			Danio	*D. devario*
		Psilorhynchidae	*Psilorhynchus*	*P. balitora*
		Nemacheilidae	*Acanthocobotis*	*A. botia*

			Schistura	*S. savona*
2.	Siluriformes	Bagridae	*Mystus*	*M. bleekeri*
		Sisoridae	*Erethistes*	*E. pussilus*
		Heteropneustidae	*Heteropneustes*	*H. fossilis*
3.	Beloniformes	Belonidae	*Xenentodon*	*X. cancila*
4.	Synbranchiformes	Mastacembelidae	*Mastacembelus*	*M. armatus*
5.	Anabantiformes	Channidae	*Channa*	*C. punctata*

Order Cypriniformes comprised of three families: Cyprinidae, Psilorhynchidae, and Nemacheilidae with 18 species and order Siluriformes comprised of three families: Bagridae, Sisoridae and Heteropneustidae with three species followed by Synbranchiformes comprised of one family: Mastacembelidae with single species, Order Beloniformes comprised of only one family: Belonidae with one pecies and Order Anabantiformes comprised of single family: Channidae with single species (Figs. 2, 3)

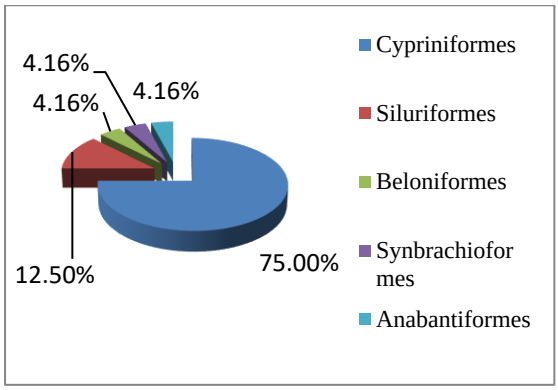


Figure 2. Order wise fish distribution in Babai River**.**

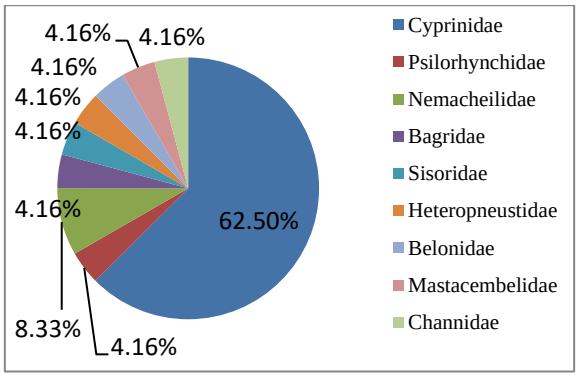


Figure 3. Family wise fish distribution in Babai River

Diversity status

The value of Shannon Wiener diversity index (H′), Species richness (d) and Evenness (E) were calculated according to each station and season. The highest Shannon Wiener diversity index was found in station III (2.62) and lowest value was found in station I (2.36). The Margalef Species richness value was observed maximum at station II (7.89) where as lowest value was observed (6.94) at station I. Evenness index was found to be highest at station III (2.01) and lowest at station II (1.88). Similarly, the highest Shannon Wiener diversity index was found in winter (2.62) followed by Autumn (2.40) and lowest value was found in spring (2.28). The Margalef Species richness value was observed maximum in Winter (7.83) followed by Autumn (7.76) where as lowest value was observed (7.67) in Spring. Evenness index was also found to be highest in winter (1.98), (1.81) in Autumn and lowest in Spring (1.74) (Figs. 4 and 5).

Figure 4. Spatial variation of species diversity index of Babai River.

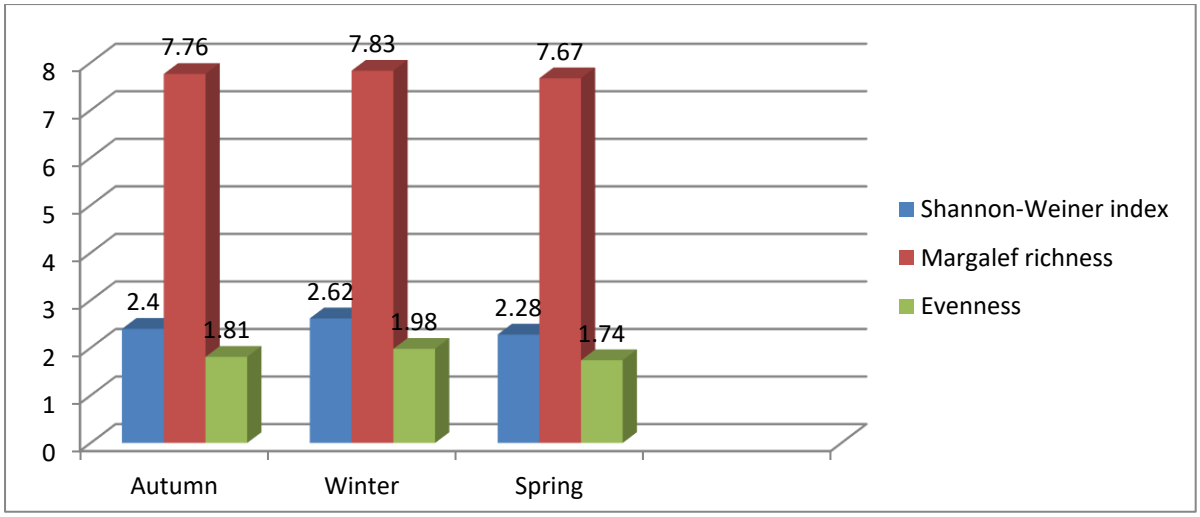


Figure 5. Temporal variation of species diversity index of Babai River.

Environmental variables, sites, seasons and fish species relationship

The result obtained after the redundancy analysis (RDA) was plotted in Figure 6. The vector length of environmental variables pointed out importance of that variable. The environmental variables of water velocity, free-carbon dioxide, pH and water temperature were found to be pivotal variables to shape the fish assemblage structure of Babai River. Dissolved oxygen and water velocity are associated with the occurrence of C19, C12 *and* C11 and showed positive correlation with site C and A. The value of pH, free carbon-dioxide and water temperature are associated with C7, C5, C8 and C2 and also showed positive correlation with site B and D.

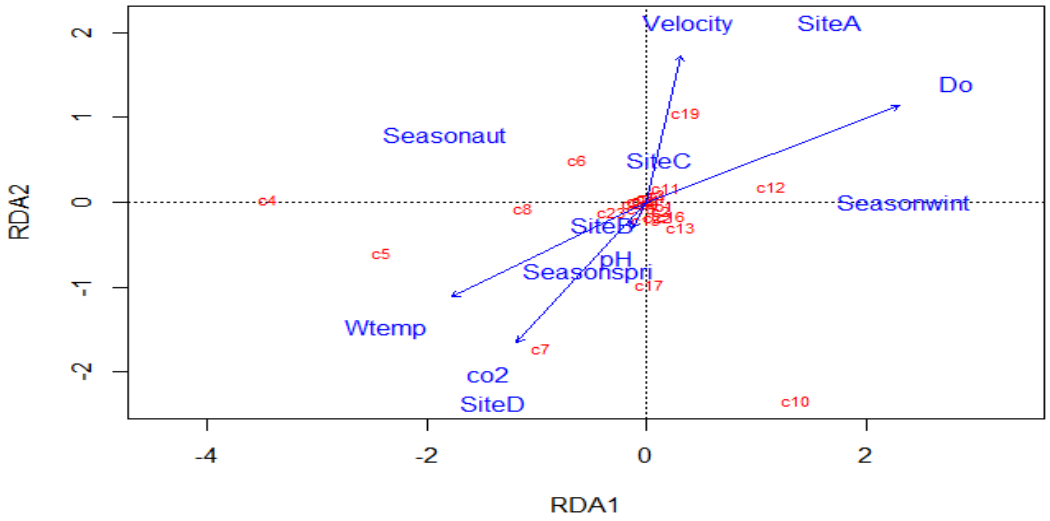


Figure 6: RDA analysis of species seasons, sites and environmental variables. (for species code see table 1) (Do = dissolved Oxygen, co_2 = Free carbon-dioxide, Wtemp = water temperature,wint = Winter, spr = Spring, aut = Autumn).

pvCluster analysis of fish species of Babai River

Hierarchial clustered dendrogram of fish species from the Babai River, black and bold colored number represents the cluster number, red represents probability of Automatic Unbiased (AU) value and blue colored number represents Bootstrap Probability (BP) value. AU value > or = 95 represents significant cluster.

Altogether twenty two cluster groups were formed with four major clusters (19, 15, 17 and 22) (Fig. 7). The first cluster group is formed

with four significant sub-cluster groups. The second cluster number 17 has also different sub groups and all the sub groups were formed significant cluster group. The cluster number 20 delineated that the cluster number 15 and 17 have no significant correlation. The third cluster group 19 is formed with four significant sub cluster groups. The last cluster group 22 has six sub cluster groups and all sub-groups showed significant distance correlation to each other.

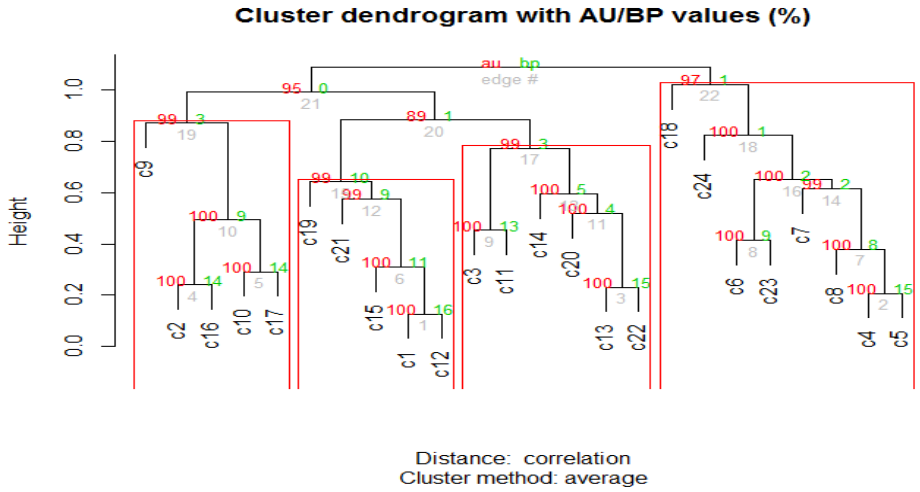


Figure 7. Dendrogram of cluster analysis comparing fish species on the basis of fish assemblage. (for species code see table 1).

Discussion

A total of 24 fish species were reported from four different sampling sites of Babai River. Among 24 species, *Puntius sophore* was the domineering species which comprises 17.40% followed by *Barilius bendelensis* 15.18%. Shrestha (2019) recorded 252 fish species which includes 15 orders, 35 families and 104 genera. Cypriniformes is the most dominating order which comprised 135 species. Majority of the fish species from the River fall under the order Cypriniformes, this is the largest order of fresh water fishes which include 2,422 species (Nelson, 1948). The present study also revealed Cypriniformes as the dominating order with 18 species which is 75% of total fish species followed by Siluriformes (3 species) 12.5%. The result of this research is also similar to the findings of Limbu *et al*. (2016), Limbu *et al*. (2019a), Limbu and Gupta (2019b), Limbu *et al.* (2018) where they have reported order Cypriniformes as a dominanted order from Ratuwa, and Bakraha River. *Barilius modestus and Barilius barna* were not found from site A throughout the study period. It might be due to the presence of stagnant water at site A. The sampling site A is near to human settlement area and thus, exposed to pollution and overfishing, accounted to only 13.9% of total catch. *Labeo fimbriatus* and *Danio devario*, were recorded from Station B, C and D. It might be due to favorable environmental condition at these sites. *Mastacembelus armatus* was recorded from under the rocks, stones from site B and D. *Heteropnestus fossilis* and *Channa punctata* were caught from nearby ditches and edges. *Erethistes pussilus, Tor putitora, Psilorhynchus balitora* and *Schistura savon-a* were sparsely found throughout the study.

Puntius sophore, Barilius bendelensis, Puntius terio and *Puntius ticto* were rifely recorded from all four sampling sites which comprised 17.40%, 15.18%, 11.41% and 10.44% of total catch respectively. Similarly, other *Puntius* species like *Puntius chola* and *Puntius conchonius* were also found ubiquitously from all four sites. These fishes were found near the bottom along with aquatic vegetations. *Garra gotyla* was caught from slightly dull running water from all four sites but only comprises 1.06% of total catch and is considered as the hill stream fish with special adaptive modification of their barbels, lips and adhesive discs. *Garra rupecula* was recorded from site B, C and shows almost similar habitat status as that of *Garra gotyla*.

Barilius barila, Barilius shacra were also recorded from all four sites and has almost similar habitat with *Barilius bendelensis*.

Acanthocobotis botia was also reported all four sites from the edges of the river. This fish prefer to hide below the sand and small algae of stagnant water. Cat fish *Mystus bleekeri* is another fish species recorded from sites A, B and C during present field visit. This fish was caught from stagnant as well as moderately running water. Fish with special modification of mouth teeth, *Xenentodon cancila* was recorded from all stations except station D with only 0.77% of total catch

Most of the fishes were recorded from all three seasons. *Puntius sophore* was found in all three seasons during the present field visit and is the most common and dominating species of Babai River. Highest number of individuals was recorded in autumn (February) and least number of individuals was recorded in Spring (May). *Barilius bendelensis, Puntius terio* and *Puntius ticto* were found in all three seasons; autumn, winter and spring. *Barilius bendelensis* had highest catch number in winter whereas *Puntius terio and Puntius ticto* in autumn. Similarly, *Garra gotyla* and *Garra rupecula* were also reported from all three seasons. *Erethistes pussilus* reported only in autumn with only 0.096% of total catch is very rare species. Likewise, *Channa punctata* recorded in spring only was also very rare species with only 0.19% of total catch in present study.

Acanthocobotis botia had the highest number of catch composition in autumn while same number of individuals was caught in Winter and Spring. *Puntius chola, Puntius conchonius, Barilius barila, Barilius modestus, Danio devario* and *Mystus bleekeri* were found in all three seasons with the common status. *Barilius shacra* was recorded from winter only but not from autumn and spring. Present study shows that *Tor putitora* is a most rare species as it has only 0.19% of total catch and recorded in winter and autumn. *Heteropnestus fossilis* was recorded in spring and winter with very low catch percentage 0.19 and considered as a rare species. *Labeo fimbriatus* was found in winter and spring while *Barilius barna* was recorded in all three seasons. *Mastacembelus armatus* was recorded from all two seasons except winter. *Xenentodon cancila, Schistura savona* and *Psilorhynchus balitora* were recorded in all three seasons with only 0.77%, 0.38% and 0.96% of total catch. The analysis of similarity (ANOSIM) vindicated significance difference in spatial variation (R=0.704, P=0.001) but no significance difference in temporal variation (R=-0.22, P=0.99) which is similar with Yan *et al.* (2010).

A biodiversity index seeks to characterize the diversity of sample or community by a single number (Magurran, 1988). The concept of the species diversity involves two components: the number of species or richness and the distribution of individuals among species. However, the formal treatment of the concept and its measurement is complex (Williamson, 1973). Highest Shannon-Weiner diversity index was found in site C and Winter where lowest was observed at site A and during Summer.

Highest seasonal Shannon Weiner diversity index value was found in winter (2.62) and lowest value in Spring (2.28) and in case of station highest diversity index was found in station C (2.62) and lowest value was found in station A (2.36). The maximum margalef richness value was also observed in winter (7.83) whereas minimum value was observed in spring (7.67) and in case of station, maximum margalef value was observed at station B (7.89) and minimum value was observed in station A (6.94). Similarly evenness index was found to be highest in winter (1.98) and lowest in spring (1.74) and in case of station, higher evenness value was observed at station C (2.01) and minimum was recorded at station B (1.88). In diversity index (H'), Evenness (E) and Mergalef richness (d), there was no significant difference observed. Therefore, it may be concluded that the seasonal difference in species diversity is a common phenomenon in the studied area.

The ordination plot revealed that water temperature, free carbon-dioxide (FCO2) and pH were found significantly correlated and showed negative relation with dissolved oxygen (DO) and water velocity. Water temperature and free carbon dioxide are strongly correlated with site D. High values of CO_2 are strongly associated with *Puntius ticto.* High value of dissolved oxygen (DO) and water velocity is associated with species like *Barilius shacra* and *Mystus bleekeri. Puntius sophore* is the dominating species of present study area and found maximum in autumn. Ordination plot also shows that Velocity and dissolved oxygen are strongly correlated with each other. It means that when water velocity increases DO also increases. *Danio devario* and *Acanthocobotis botia* both are associated with season spring and winter.

1. *Labeo fimbriatus*

2. *Garra gotyla gotyla*

3. *Garra rupecula*

4. *Puntius terio*

5. *Puntius chola*

6. *Puntius sophore*

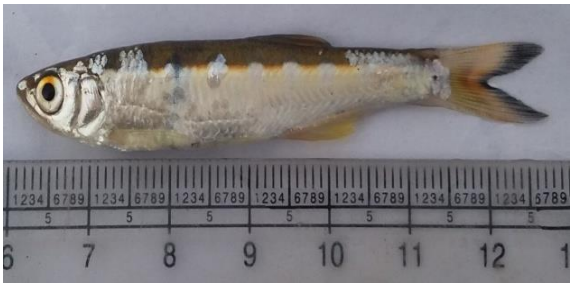
7. *Puntius ticto*

8. *Puntius c*

9. *Tor putitora*

10. *Barilius bendelensis*

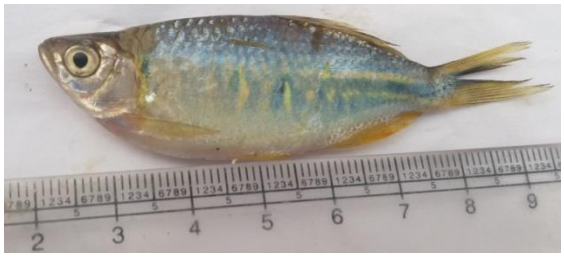
11. *Barilius modestus*



12. *Barilius barila*

13. *Barilius shacra*

14. *Barilius barna*



15. *Danio devario*

16. *Psilorhynchus balitora*

17. *Acanthocobotis botia*

18. *Schistura savona*

19. *Mystus bleekeri*

20. *Erethistes pussilus*

21. *Heteropnestus fossils*

22. *Xenentododon cancila*

23. *Mastacembelus armatus* 24. *Channa punctata*

Figure 8. Fishes of Babai River.

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References

Blanc, L., C. Aliaume, A. Zerbi, Lasserre and G. Lassare. 2001. Spatial and temporal co-structure analyses between ichthyofauna and environment: an example in the tropics. *Life Sci **324**: 635–645.*

Chowdhury, M.S.N., M.S. Hossain, N.G. Das and P. Barua. 2010. Environmental variables and fisheries diversity of the Naaf river estuary, Bangladesh. *Journal of Coastal Conservation **15 (1)***, 163– 180.

Clarke, K.R. 1993. Non parametric multivariate analyses of changes in community structure. *Australian Journal of Ecology **18**, 11743*

Conversi, A., V. Dakos, A. Gardmark, S. Ling, C. Folke, P.J. Mumby, C. Greene, et al. 2015. A holistic view of marine regime shifts. Philosophical Transactions of the Royal Society B: *Biological Sciences, **370**: 20130279.*

Gauch, H.G.J. 1982. Multivariate analysis in community ecology. Cambridge: Cambridge University Press.

Gorman, O.T., 1988. The dynamics of habitat use in a guild of Ozark minnows. Ecol. Monogr.

Grossman, G.D, R.E. Ratajczak, M. Crawford, and M.C. Freeman. 1998. Assemblage organization in stream fishes: effects of environmental variation and interspecific interactions. *EcolMonog **68(3)**:* 395-420.

Harvey, B.C and A.J. Stewart. 1991. Fish size and habitat depth relationship in headwater streams. *Oecologia **87***: 336-342.

Hossain, M.S, N.G. Das, S. Sarker, and M.Z. Rahaman. 2012. Fish diversity and habitat relationship with Environmental vriables at Meghna river estuary, Bangladesh. *Egyptian Journal of Aquatic Research. **38,*** 213-226.

Jayaram, K.C. 2010. The freshwater fishes of Indian region. Narendra Publishing House, Delhi, India, 614.

Kadye, W.T, C.H.D. Magadza, N.A.G. Moyo, and S. Kativu. 2008. Stream fish assemblages in relation to environmental factors on a montane plateau. *Environ Biol Fish **83**:*417-428.

Limbu, J.H., C.B. Baniya, and A. Prasad. 2019a. Spatio-Temporal Variation of Fish Assemblages in Ratuwa River, Ilam, Nepal. *J Ecol & Nat Resour **3(3):*** 000168.

Limbu, J.H., N. Chapagain, S. Gupta and S. Sunuwar. 2019b. Review on fish diversity of eastern Nepal. *International Journal of Fisheries and Aquatic Studies. 7(3):* 177-181.

Limbu, J.H and S. Gupta. 2019. Fish diversity of Damak and lower Terai region of Ratuwa River of Jhapa district, Nepal. *International Journal of Fauna and Biological studies. **6**(01-*04)

Limbu, J.H., A. Prasad, and Om.H. Shrestha. 2018. Ichthyofaunal diversity of Bakraha River of Morang district, Nepal. *International Journal of Fisheries and Aquatic Studies. **6(5):*** 267-271.

Limbu, J.H., G.S. Acharya, and Om.H. Shrestha. 2016. A brief report on ichthyofaunal diversity of Dewmai Khola of Ilam district, Nepal. *J. Nat. Hist Mus. Vol. **30.*** 312-317.

Magalhaes M.F, D.C. Batalha, M.J. Collares-Pereora. 2002. Gradients in stream fish assemblages across a Mediterranean landscape: contributions of environmental factors and spatial structure. *Freshw Biol **47:** 1015-1031.*

Magurran, A.E. 1988. Ecological Diversity and its Measurement. Princeton University Press, Princeton.

McGill, B. J, B,J. Enquist, E. Weiher, and M. Westoby. 2006. Rebuilding community ecology from functional traits. *Trends in Ecology and Evolution, **21***: 178–185.

Oksanen, J, F.G. Blanchet, R. Kindt, P. Legendre, and P.R. Minchin. 2019. Vegan: Community ecology package. R package version 2: 3-1.

Perry, A..L., Low, P.J., Ellis, J.R and Reynolds, J.D. 2005. Climate Change and Distribution Shifts in Marine Fishes. *Science*, ***308:*** 1912–1915.

Pessanha, A.L.M and F.G. Araujo. 2003. Spatial, temporal and diel variations of fish assemblages at two sandy beaches in the Sepetiba Bay, Rio de Janeiro, Brazil. *Estuar. Coast. Shelf Sci*. ***57,*** 817–828.

R Core Team. 2018. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Rouyer, T, J.M. Fromentin F. Menard, B. Cazelles, K. Briand, R. Pianet, B. Planque. 2008. Complex interplays among population dynamics, environmental forcing, and exploitation in fisheries. Proceedings of the National Academy of Sciences of the United *States of America*, ***105:*** 5420–5425.

.Shrestha, T.K. 2019. Ichthyology of Nepal, a study of fishes of the Himalayan waters. Prism Color Scanning and Press Supportive Pvt. Ltd, Kathmandu, Nepal.

Suzuki, R and H. Shimoddaira. 2019. Pvclust: Hierarchical Clustering with P-Values via Multiscale Bootstrap Resampling. R package version 2.0-0.

Talwar, P.K and Jhingram, A.G., 1991. Inland fishes of Indai andAdjacent countries. Oxford and IBH Publishing Co. India, (I, II):1158.

Ter Break, C.J.F. 1986. Canonical correspondence analysis-a new eigenvector technique for multivariate direct gradient analysis. *Ecology.* ***67(5):***1167–1179.

Williamson, M. 1973. Species diversity in ecological communities. In: Bartlett, M.S., Horns, R.W. (Eds). The Mathmatical Theory of the Dynamics of Biological Populations. Academic, London, pp. 325-336.

Yan, Y, H.E. Shan, C.H.U. Ling, X. Xiuying, J.I.A. anju, T.A.O. Juan and C. Yifeng. 2010. Spatial andtemporal variation of fish assemblages in a subtropical small stream of the Huangshan Mountain. *Current Zoology:* ***56(6):*** 670-677.

Yu, S.L and T.W. Lee. 2002. Habitat preference of the stream fish, *Sinogastromyzon puliensis. Zool Stud* ***41:*** 183-187.